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(*Recognosce notum, ignotum inspice*)

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A LUMINOUS AGARIC (*PLEUROTUS SP.*) FROM SOUTH BURMA

By S. R. BOSE

(*Professor of Botany, Carmichael Medical College, Calcutta, India*)

(With 3 Text-figures)

Pieces of luminous wood were obtained from Tenasserim in South Burma in August 1931, and August 1933, through the kindness of Mr P. T. Russell, Superintendent of the Cinchona Cultivation in South Burma under the Botanical Survey of India. In October 1932, from the same place, other pieces were obtained through the kindness of Mr K. P. Biswas, Curator of the Botanical Gardens Herbarium, Shibpur, on a collecting tour to South Burma. The wood belonged chiefly to dead stumps of *Lagerstroemia* sp. (Lythraceae) and *Pentace burmannica* (Tiliaceae). The luminosity had almost failed when the wood reached my hands at Calcutta. The pieces were at once wrapped with moist cotton-wool and kept in a tray with a little water. The light increased in the course of a few days, and a number of tiny fructifications of a *Pleurotus* developed on some of them in the course of a month. The fructifications were luminous all over, that is to say, the stalks as well as both surfaces were equally luminous. The vegetative mycelium in the wood giving rise to these fructifications was also luminous; hence pieces of wood infected with fungal hyphae looked very bright in the dark. In this respect the fructifications differed from those in Buller's list(4) of twelve luminous species of *Pleurotus*, in which only the fruit body is luminous.

Since the *Pleurotus* examined does not exactly agree with the descriptions of luminous species from Australia, the Philippine Islands, Japan and America (*P. candescens* F.V.M., *P. Gardneri* Berk., *P. illuminans* F.V.M., *P. Lampas* Berk., *P. nidiformis* Berk., *P. phosphoreus* Berk.(=*P. olearius* DC.), *P. japonicus* Kaw., *P. facifer* B. & C., *P. igneus* Rumph., *P. noctilucens* Lév. and *P. Prometheus* B. & C.), a detailed description of the species is given with figures of its two surfaces (Figs. 1 and 2).

Pileus: Kidney-shaped with centre depressed, 10 × 8 mm., quite smooth, of pale tawny colour turning to white at the margin, upper surface marked with fine radiating anastomosing lines. Margin involute, flesh thin.

Stalk: Lateral, 8 mm. long, white, dilated above, quite smooth, solid and rigid.

Hymenial surface: Pure white, gills decurrent, edge entire, tramal hyphae running divergently parallel.

Basidia: 12–16 × 6–8 μ. Spores white, oval, 2–3 × 4–5 μ. Cystidia none. Fructifications usually do not revive in moist condition when once fully dried.

From a spore print on a sterilised clean glass slide a number of spores were removed aseptically to tubes of malt-extract agar kept at room temperature in diffused light. In three to four days fine white mycelium developed and covered the slants in a further period

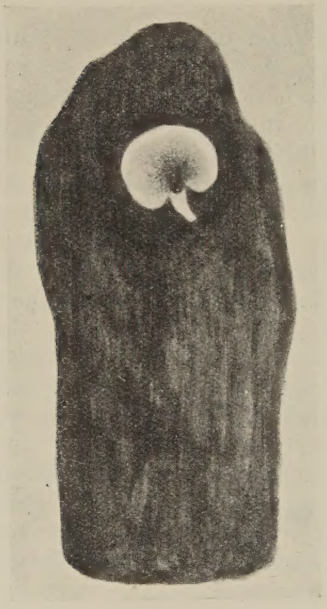

Fig. 1. Upper surface of luminous *Pleurotus* sp.

Fig. 2. Hymenial surface of luminous *Pleurotus* sp.

of ten days. Several subcultures were grown on sterilised bread media in Petri dishes, tubes and Erlenmeyer flasks, and on sterilised potato slabs within Roux tubes and on sterilised blocks of the luminous wood within Roux tubes, with a little water at the constricted end. Tissue culture from a clean piece of the sporophore was also successful in bread as well as in malt-extract agar medium. In liquid media of 1 per cent. peptone and 2 per cent. glucose the growth was mostly submerged, the colour of the media changing to brownish in the course of time.

The mycelium in these artificial cultures, as long as it is pure white, gives out a soft greenish light. A spectroscopic analysis of the emitted

light kindly carried out by Dr P. Krishnamurrti, with the help of a quartz spectroscope, under the direction of Prof. Sir C. V. Raman, F.R.S., shows that the rays are mostly in the greenish region—5400–5000 A. When the white mycelium becomes brown, in thirty to forty days, in culture flasks and tubes, it no longer gives out any light. The browning of the white hyphae occurs in patches and it takes a long time (about two months) to become uniform deep brown all over the cultures. The luminosity in artificial cultures has been kept more than two years by continual subcultures in fresh bread media. Wounding the mycelium with a sterilised needle at once

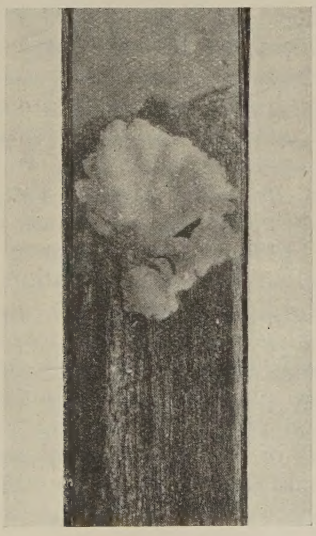

Fig. 3. Fructification in artificial culture on sterilised luminous wood block within Roux tube (portion of the tube shown).

increases the luminosity and causes fresh growth of young white hyphae over the old ones. In bread media in flasks the hyphae ultimately exude copious drops of white liquid which gradually turn brownish to black. In older cultures the hyphae form compact strands here and there, and clamp connections are abundant. In this condition the hyphae become submerged and form brownish patches here and there; here also only the white areas give out light. Fructifications have not yet appeared in these bread media during these two years, but on sterilised wood blocks tiny white fructifications appear in six to eight months (Fig. 3). The best vegetative growth takes place in sterilised bread media and in potato slabs at room temperature.

Bothe's (3) experience with the culture of luminous *Mycena tintinnabulum* was almost the same except that the light of his specimen was white and not greenish, and that he could not get any fructification in artificial culture.

The effect of sunlight on the luminosity of the fungus was tested by exposing the hyphae within the culture vessels directly to the rays of the sun by removing the cotton plugs. Closed vessels were also exposed to the sun. Increase in luminosity, usually of a temporary nature, was observed only when the hyphae had been directly exposed to the sun's rays. Luminosity did not fail entirely even when a culture on bread medium was kept in entire darkness for a month and a half. On the other hand light could develop well in the course of a month in a flask inoculated with the luminous mycelium, though it was kept in total darkness in an incubator at 22° C. This shows that luminosity here is continuous and quite independent of previous illumination of the fungus, as has been remarked by Harvey ((6), pp. 68–9) in bacteria and fungi in general. In this respect there is a great difference from the behaviour of the luminous wood (of *Sterculia* sp.) that I (2) received from Buxa Duars in Jalpaiguri (Bengal) in September 1929. There the luminescence was distinctly intermittent, depending on the general stimulation of the living hyphae in the presence of the sunlight; Perkins's (10) experience with the light of glowworms, which glow only by night, was almost the same. He concluded that external light was probably not needed for storage on the lines of chemical phosphorescence but necessary to stimulate natural conditions. Unfortunately, light did not develop in cultures (on a bread medium) of the fungus from the luminous wood of Jalpaiguri; neither could the fungus be identified, since the cultures never fruited but continued long in the vegetative condition, as did the mycelium X of Molisch (9).

No satisfactory explanation can be given as yet of the utility of the luminosity to the fungus itself. On the other hand there is every chance that the glow of light in the dark may attract a number of insects or mites which may cause injury to the fungus. Recently, Gurwitsch (5) and his collaborators have reported emission of invisible mitogenetic rays falling in the ultra-violet region of the spectrum from a great variety of cells and tissues; according to them the most powerful source of radiation appears to be the material which is in a high state of mitotic, metabolic, nervous or mechanical activity. Potozky and Braunstein (11) claim that in a variety of chemical oxidations ultra-violet light of the same wave-length as the mitogenetic rays of Gurwitsch may be produced. The luminescence of the fungus described here depends largely on the access of oxygen, but in contrast to Gurwitsch's radiation the rays are mostly in the greenish region of the spectrum.

Attempts to extract enzymes in luminous condition from the mycelia, using Harvey's methods (6), were unsuccessful. Neither did the mixing together of extracellular and intracellular enzymes produce any light in the solution.

The response of the luminous *Pleurotus* to oxygen, hydrogen, nitrogen and carbon dioxide was the same as that of luminous leaves and stalks from different parts of Bengal (1).

Subsequently, in November and December 1933, I obtained from Khandala, Bombay, pieces of dead wood, containing fungal hyphae sent four months after collection by Mr K. J. Kabraji of the Poona Meteorological Department, Bombay. Luminosity had almost disappeared when the pieces of wood reached me, but could be revived after soaking with distilled water for two or three days. I am told that such luminous twigs are very common on the slopes of the Western Ghats of the Bombay Presidency, particularly the Umri hilltop at about 2000 ft. elevation. Sterilised bread media in flasks were at once inoculated with a small piece aseptically removed from the inside of the luminous twigs. Subcultures in the same medium gave pure white growth of the mycelium fully covering the outer surface of the bread in the course of a week. Subcultures have also been started on sterilised pieces of luminous twigs within Roux tubes with a little water at the constricted end. Light has not developed as yet in these artificial cultures; neither has any fructification appeared. Consequently the identity of the fungus causing the luminosity has not been established. Direct exposure to sunlight increases the luminosity of the luminous twigs, as in luminous wood of *Sterculia* reported from Buxa Duars (2).

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OBSERVATIONS ON *FOMES POMACEUS* (PERS.) BIG. & GUILL. INFECTING PLUM TREES

By EILEEN FISHER
(*Botany School, Cambridge*)

(With 4 Text figures)

Plum trees growing in Cambridgeshire are frequently attacked by the fungus *Fomes pomaceus* (Pers.) Big. & Guill. Although the variety Pershore is generally most susceptible, on one plantation at Histon a large number of trees belonging to the variety Victoria were found to be heavily infected.

Review of previous work

Although the literature concerning wood-rotting fungi is so extensive, *Fomes pomaceus* seems to have escaped the attention of most investigators. Baxter (1) has studied a resupinate form of *F. pomaceus* occurring chiefly on *Crataegus*, but also on *Prunus americana*; and this is the first record of any cultural study of the fungus.

Owing to the similarity of decay produced by *Fomes pomaceus* and by *F. igniarius* (Linn.) Fr., special attention has been paid to references concerning the latter species.

With both of these fungi a "gumming" of the wood in the early stages of decay is followed by a "white-rot." The general opinion of those who have studied these species seems to be that the "white-rot" is due to delignification of the wood, although this suggestion is not supported by those investigators who have tackled the problem from a chemical standpoint.

More recently Campbell (4, 5) has made a chemical examination of several kinds of "white-rot," and he has shown that the characteristic feature of this type of decay concerns the pentosan constituents of the wood, and that some lignin may remain even after the development of the typical white colour.

In 1878, Hartig stated that the delignifying action of *Polyporus igniarius* was first directed towards the inner part of the wall of the wood fibres, dissolution of the resulting cellulose layer following later.

Spaulding (17) found "an inner supernumerary layer of cellulose" lining the healthy wood fibres of *Populus tremuloides*, while in wood infected with *Fomes igniarius* removal of this cellulose layer preceded delignification of the middle lamella region.

In 1909, von Schrenk and Spaulding stated that one of the principal effects of *Fomes igniarius* on the wood of the host was "solution of the lignin elements of the cell wall."

However, by chemical analysis of aspen wood infected with *F. igniarius*, Johnson and Hovey(12) have since shown that it is the cellulose rather than the lignin constituent of the wood which is attacked by this fungus.

In 1925, Baxter included in his study of the "hard-wood heart-rotting fungi" both *F. igniarius* and a strain of *F. pomaceus*. From microchemical tests he concluded that both these fungi had a delignifying action on the elements of the wood, and that the decay produced by *F. igniarius* might be distinguished by the development of black lines limiting the infected zone.

Observations on the germination and longevity of the spores

An inspection was made of infected plum trees growing at Meldreth, Cambridgeshire, at approximately monthly intervals from November 1931 to May 1932.

Fructifications of the fungus were gathered throughout this period, and spore deposits were obtained in the laboratory by suspending the fruit bodies over sterile microscope slides. After about twenty-four hours a thick white deposit of spores was usually obtained in this way.

White(19) from observations on spore deposits collected in the field from fructifications of *F. applanatus* found that spore discharge, which began in early spring, was brought to an end by heavy frosts in late autumn. However, he obtained spore deposits from detached fructifications during the winter, and this he attributed to loss of surface adhesion on the part of spores lodged in the pores.

In the present investigation the most copious spore deposits of *F. pomaceus* were obtained from fructifications collected during the last week of November, although scanty deposits were obtained throughout the winter. The spore-covered slides were kept in a closed box and the germinability of the spores was tested from time to time. For this purpose a spore suspension was made in sterile tap water. This was then painted with a camel-hair brush over a film of Dox's agar on a slide, which was kept in a sterile Petri dish. Microscopic examination of the spores was made each day. This method, which proved very satisfactory for studying spore germination, is essentially the same as that used by Hirt(11) in his study of *Polyporus gilvus*, but it was found that the use of slides facilitated microscopic examination. Vernon(18) has described a method for studying fungal

growth microscopically, in which the fungus is grown under a cover-slip, and the medium takes the form of a small disc of agar. This method proved unsatisfactory for *Fomes pomaceus*.

The latent period of germination in these spores seems to be long, as in no case was any sign of activity noticed for at least seventy-two hours. White (19) and Hirt (11) found this period to be much shorter for spores of *F. applanatus* and *Polyporus gilvus* respectively; in these latter fungi, water alone was sufficient to induce germination of the spores of *Fomes pomaceus*, and a nutrient medium, which Buller (3) found necessary with spores of *Polyporus squamosus*, was not required. The first visible stage of this process was the development of a single unbranched non-septate germ tube, followed within approximately twenty-four hours by the appearance of a second germ tube arising from the opposite end of the spore.

It was found in this way that some spores of *Fomes pomaceus* retained their germinability for a period of twenty-four weeks.

Cultural study of the fungus

Cultures were obtained from spores of *F. pomaceus*, and the cultures isolated from decayed wood were compared with them and found to be identical.

Isolations from diseased wood were made in the following manner: A length, approximately 2–3 in., of a diseased branch was chiselled into small triangular segments. By passing the wood segment through a flame the surface was sterilised, and then shavings, cut with a sterile razor from the junction of decayed and healthy wood, were plated out on to a nutrient medium such as prune agar. About five days later, fungal colonies developed, which after subculturing on to plain agar were transferred to tubes containing some suitable nutrient medium. Sterilised plum wood blocks formed a very convenient medium for the growth of the fungus. The medium used seemed to have little effect on the type of growth, which remained the same on malt agar, Dox's agar, and plum wood blocks. The mycelium is of a woolly nature due to dense development of aerial hyphae, and is colourless in the early stages of its growth, but later darkens to "cinnamon buff" (Ridgway (13)).

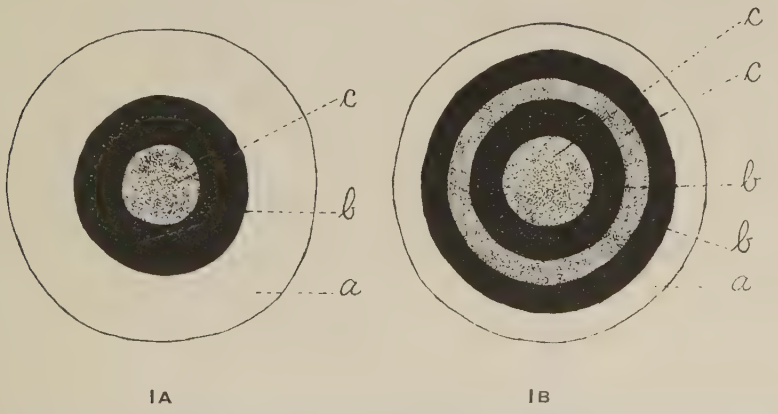
In *F. pomaceus* forma *Crataegi* isolated by Baxter (1) the mycelium is apparently darker in colour, and when grown on malt extract agar it becomes "cinnamon brown" and later "snuff brown" (Ridgway).

The mycelium of *F. pomaceus* retains its vitality for a long time, a fresh culture having been established from one on a plum wood block after three and a half years.

Effect of the fungus on plum wood

Examination of the cut surface of badly infected branches revealed a white crumbling mass in the central region of the heart-wood, separated from the outer healthy wood by a brown "gummy" zone (Fig. 1 A). In some specimens the "gummy" and the more advanced stage of decay or "white-rot" alternated, as illustrated in Fig. 1 B.

Microscopic examination was made of the wood from healthy, "gummy," and "white-rot" zones respectively. Sections of the last two zones, stained with safranin and picro-aniline blue (Cartwright (6)), or with safranin and light green, clearly showed fungal hyphae, which were absent from the healthy wood (Fig. 2).



1A 1B

Fig. 1 A and B. Diagrams illustrating the appearance of the wood of the cut ends of two plum branches infected with *Fomes pomaceus*. *a*, zone of healthy wood; *b*, "gummy" zone; *c*, "white-rot" zone.

From macroscopic observation, it is evident that in the early stage of decay the wood remains quite firm and may be distinguished from that in the healthy condition only by its brown colour, due to the accumulation of large quantities of gum in the vessels and medullary-ray cells. From a comparison of sections from healthy and "gummy" regions, treated with iodine, the development of gum in the latter would appear to be connected with the disappearance of starch from the medullary-ray cells. This is contrary to the observations by Hartig (10) on the effect of *Polyporus igniarius* on oak wood, for he found the disappearance of the starch grains to be deferred until after the removal of the lining layer of the fibres. On the other hand, my observations support the theory of gum formation put forward by

Brooks and Storey (2) in connection with silver-leaf disease of plum trees caused by *Stereum purpureum*.

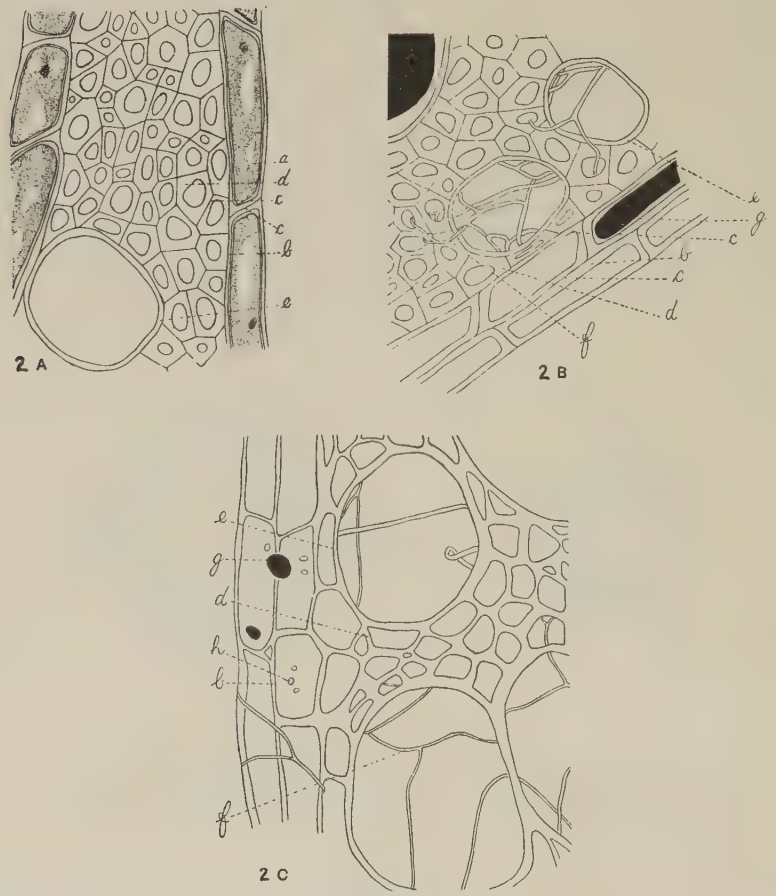


Fig. 2. Transverse sections of plum wood infected with *Fomes pomaceus*. ×312. Stained with safranin and picro-aniline blue. A, *healthy zone*, showing thick-walled wood fibres and protoplasmic contents of the medullary-ray cells. B, "*gummy*" *region*, showing fungal hyphae and large quantities of gum. C, "*white-rot*" *zone*, showing thinner-walled fibres and empty medullary-ray cells. *a*, protoplasmic contents of medullary-ray cell staining blue; *b*, wall of medullary-ray cell; *c*, middle lamella; *d*, wall of fibre; *e*, wall of vessel; *f*, fungal hypha staining blue; *g*, gum; *h*, simple pit. The walls of the wood elements take up the safranin stain throughout, especially in the middle lamella.

Microscopic examination revealed that the more advanced stage of decay, when the wood becomes white and soft, is characterised not only by a disappearance of gum but also by reduction in thickness of the fibre walls.

Harlow(8, 9) has drawn attention to the unreliability of microchemical methods as a means of cell-wall analysis; and Hirt(11) in his investigation of the decay caused by *Polyporus gilvus* regarded results derived from staining reactions as significant only when supported by chemical analysis.

In the present investigation, which was completed in Australia, the amount of decayed wood available was limited, so it was not possible for a chemical analysis to be made. However, some indication of the changes taking place can be ascertained by reviewing the results of some of the more reliable microchemical tests when applied to healthy and decayed wood.

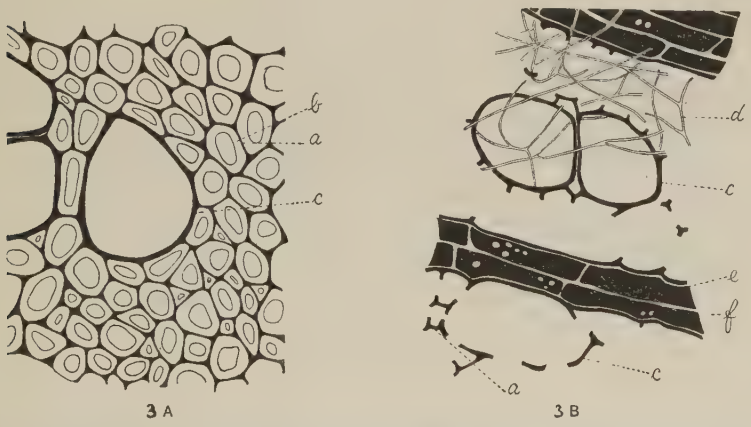


Fig. 3. Transverse sections of plum wood. Stained with safranin and light green. A, healthy wood showing differentiation of fibre wall into two distinct layers. × 312. B, wood in advanced "white-rot" stage of decay caused by *Fomes pomaceus*. × 188. *a*, middle lamella of fibre wall staining red; *b*, lining layer of fibre wall staining green; *c*, wall of vessel staining red throughout; *d*, fungal hypha staining green; *e*, wall of medullary-ray cell staining red throughout; *f*, simple pit.

Sections of healthy wood stained overnight with 1 per cent. safranin in 50 per cent. alcohol, and counter-stained with light green for thirty minutes, showed clearly that the fibres were provided with a thick lining layer which stains green. This is apparently quite distinct in its constitution from that of the middle lamella of these elements, and from the entire wall of the other elements, vessels and medullary-ray cells, which take up the safranin stain (Fig. 3A).

The application of phloroglucin and hydrochloric acid resulted in a similar differentiation of the fibre wall into two distinct layers; (i) the middle lamella, giving a bright pink reaction, which was absent from (ii), the thicker lining layer. The same pink coloration was produced in the wall of the vessels throughout its thickness, and

in the medullary-ray cells the middle lamella appeared as a clear-cut pink line in the central region of the rather lighter stained general cell wall (Fig. 4).

The same cell wall layers again became differentiated when wood sections were treated with aniline sulphate and sulphuric acid, but here the colour reaction was golden yellow. As pointed out by Crocker (7) it has been shown that these two latter colour reactions are due to aldehydes, mainly coniferyl aldehyde, which accompany the lignin.

The Mäule reaction is another very generally used test for lignin, and it is recommended by Schneider and Zimmermann (15) for use in

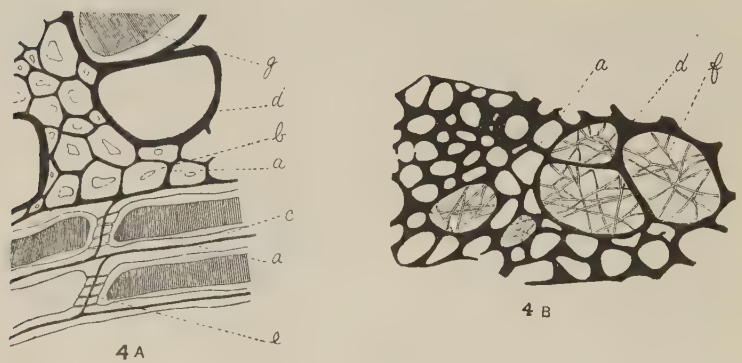


Fig. 4. Transverse sections of plum wood infected with *Fomes pomaceus*. ×312. Stained with phloroglucin. A, "*gummy*" *region*, showing the lining layer of the wall of the fibres and abundance of gum. B, "*white-rot*" stage of decay, in which the pink-staining skeleton formed by the middle lamellae of the fibres and vessels is infested with fungal hyphae. *a*, middle lamella of fibre wall, stained bright pink; *b*, lining layer of wall, unstained; *c*, wall of medullary-ray cell, stained faintly pink; *d*, wall of vessel, stained bright pink; *e*, simple pit; *f*, fungal hypha; *g*, gum.

conjunction with the phloroglucin test and as complementary to it; the colour reaction is dependent on a portion of the lignin molecule which is distinct from the chemical grouping responsible for the pink coloration with phloroglucin. Sections of healthy wood were placed in a 1 per cent. potassium permanganate solution for five minutes, then after washing with water and dilute hydrochloric acid for one to two minutes, application of a drop of ammonia produced a deep red coloration throughout the entire wall of all elements of the wood. The lining layer of the fibres did not become differentiated from the middle lamella as when treated with phloroglucin.

In view of this evidence it seems impossible to consider the lining

layer of the fibres as consisting of pure cellulose*, although sections treated with chlorzinc-iodine indicate that this compound is present in considerable quantity, as is shown by a definite blue coloration in this region. The middle lamella of the fibres and the entire wall of the vessels show no colour change at all; similarly the middle lamella of the walls of the medullary-ray cells remains unchanged, but the other parts of these walls are stained faintly blue.

Spaulding (17) used only the phloroglucin and chlorzinc-iodine tests when he described an "inner supernumerary layer of cellulose" in the wall of the wood fibres of *Populus tremuloides*.

That other substances in addition to cellulose take part in the constitution of this lining layer in plum-wood fibres at least is also indicated by the effect on it of various cellulose solvents. The action of (i) 72 per cent. sulphuric acid, (ii) ammoniacal cupric oxide, and (iii) zinc chloride dissolved in twice its weight of hydrochloric acid, was tested on sections of healthy wood, and in every case this layer of the fibre wall remained apparently unaltered, even when the sections were left soaking in the solvent for some days.

From these microchemical tests it would appear that, in healthy plum wood, the walls of the tracheae are of uniform composition, but in the fibres the lignin is more concentrated in the region of the middle lamella where it is accompanied by certain aldehydes, while in the remainder of the fibre wall the cellulose content is higher and the aldehydes usually associated with lignin are absent. In the medullary-ray cells the lignin and its associated aldehydes appear to be concentrated in the middle lamella region, the remainder of the cell wall being slightly richer in cellulose as is shown by the action of chlorzinc-iodine, although the blue colour reaction is not nearly so intense as that produced in the fibres.

Sections of plum wood infected with *Fomes pomaceus* were subjected to the above-mentioned microchemical tests and compared with those of healthy wood.

In the early "gummy" stage of decay the walls of the woody elements apparently remain unaltered, the only visible change in the wood being an accumulation of large quantities of gum, accompanied by the disappearance of starch from the medullary-ray cells.

Of the decay in the later "white-rot" stage, the outstanding

* Ritter (14), presumably from this portion of the cell wall, has isolated partially a second type of lignin which he termed "cell wall" lignin. Harlow (8, 9) attempted to confirm Ritter's results "but in no case could conclusive evidence be found of the presence of 'cell wall' lignin," and Harlow has suggested that this substance might be a mixture of "middle lamella" lignin and a certain amount of unhydrolysed cellulose.

In (8) Harlow has questioned the reliability of the Mäule reaction for purposes of microchemical analysis, attributing the red colour developed in the secondary cell wall to spreading of the "lignone chlorides" from the middle lamella.

feature was the removal of the lining layer from the wall of the fibres, leaving a framework in which the so-called "lignin colour reaction" was readily induced by the application of phloroglucin and hydrochloric acid (Fig. 4). This stage of decay appears also to be characterised by the disappearance of the gum which accumulated in the early stages of infection, leaving the medullary-ray cells quite empty, so that the simple pits on the walls of these cells become clearly visible (Figs. 2C and 3B).

It has been stated by Baxter(1) that in wood decay produced by the strain of *F. pomaceus* studied by him, the walls of the medullary-ray cells are the last to be attacked by the fungus. Observations made during the present investigation lend support to this contention. In transverse sections of plum wood in the final stages of decay, the medullary-ray cells were observed forming radiating plates, which were conspicuous even when the framework of the other woody elements showed signs of disorganisation (Fig. 3B). Also, comparison of sections taken from "gummy" and "white-rot" stages of decay respectively showed the increase in hyphal development in the latter stage to be most manifest in the medullary rays. On the other hand, von Schrenk and Spaulding(16) have found that with *F. igniarius* it is in the earlier stages of decay that the hyphae develop abundantly in the medullary-ray cells.

Plum wood in the final "white-rot" stage of decay was found to be very densely infested with fungal hyphae, which indeed formed the most striking feature when sections of such wood were examined microscopically. The hyphae become finer as decay proceeds, and this fact, which has also been noted by Hartig(10) and Buller(3) in *Polyporus igniarius* and *P. squamosus* respectively, is attributed by them to lack of oxygen and starvation of the fungus.

Softening of the wood in the "white-rot" stage of decay produced by *Fomes pomaceus* and by *F. igniarius* has been attributed by the majority of investigators to delignification. Evidence derived from the present investigation on the effect of *F. pomaceus* on plum wood does not substantiate this suggestion, as in the most advanced stages of decay the remaining skeleton of woody tissue still gave a positive reaction when lignin tests, such as phloroglucin and Mäule's method, were applied. The characteristic softening of the wood appears to be due rather to the removal of the layer lining the fibres. In so far as an accurate analysis can be made from the microchemical methods used, these fibres may be regarded as lignified, although devoid of the aldehydes which usually accompany lignin, and as being rich in cellulose. This conclusion is in agreement with the observation, made from chemical analyses by Johnson and Hovey(12), that *F. igniarius* attacks the cellulose rather than the lignin constituents of aspen wood.

Inoculation experiments

During the last weeks of 1931 and the early part of 1932 a number of Victoria and Pershore plum trees were inoculated with *F. pomaceus*. Some of the trees were growing in the open at the Botanic Garden, and others in a greenhouse at the University Farm, Cambridge.

These experiments were carried out on branches about $\frac{1}{4}$–$\frac{1}{2}$ in. in diameter with (i) spores, (ii) actively growing mycelium. In the first method a spore suspension in sterile water was made, and this, applied by means of a sterile pipette to the freshly cut end of the twig, was drawn into the wood vessels. In the second method each mycelial inoculation was made by means of a *T*-shaped incision in the stem of the tree. After the introduction of the organism and moistening with sterile water, the wound was bound up with thick black wool. Control experiments using sterile water were also made.

Unfortunately, this study of the pathogenicity of *F. pomaceus* on plum trees was terminated rather prematurely by my return to Australia, about six months after the inoculations were made. Some of the inoculated twigs were cut and examined, and although the fungus had not grown sufficiently during this period to make any accurate observations, the consequent browning of the wood appeared to extend further in the variety Pershore than in Victoria.

In March 1933, that is approximately fifteen months after the inoculations were made, those twigs which had not already been removed earlier, were cut and examined by Mr Brooks. At the end of this period branches inoculated with mycelium showed very slight spread of the fungus, and the browning of the wood extended at most for 1 in. above and 1 in. below the point of inoculation, with only one exception, a twig from a Pershore tree in which the wood exhibited discoloration for 2 in. above and 4 in. below the inoculation point. Inoculation by means of a spore suspension resulted for the most part in death of the "snag" only, *i.e.* the portion of the twig extending from its cut surface down to the nearest node below, which dies inevitably without inoculation. In two instances, however, death of the twig inoculated with a spore suspension extended below the snag; in one of these, on a Victoria tree, death extended for 2 in., while in the other, on a Pershore tree, it extended for 4 in. below the snag.

It may be concluded therefore that under the conditions which prevailed during the course of these experiments, *F. pomaceus* is only very feebly parasitic on plum trees, and that the variety Pershore is rather more susceptible than the variety Victoria. In plum plantations usually only old trees are attacked by this fungus and the progress of the disease is generally slow. In the experiments described

above it was only possible to inoculate comparatively young branches. Perhaps inoculations of older wood would have indicated a more rapid spread of the fungus.

Summary

1. The incidence of *F. pomaceus* on plum trees in the Cambridgeshire district is noted.

2. Attention is drawn to the similarity in the type of wood decay produced by *F. pomaceus* and *F. igniarius*, and an outline of the results of previous investigations on these two fungi is given.

3. Spores of *F. pomaceus* are sometimes capable of germination twenty-four weeks after liberation from the sporophores.

4. Isolations of *F. pomaceus* have been made from decayed wood, and these cultures are found to be identical with those grown from spores.

5. The cultural characters of the fungus are noted and compared with those of the strain isolated by Baxter.

6. The fungus is very resistant to desiccation; a fresh culture was obtained from one that was three and a half years old.

7. A study of the effect of the fungus on plum wood has been made, using microchemical methods.

8. The copious gum formation which is so characteristic of the early stages of decay is associated with removal of starch from the medullary-ray cells.

9. The characteristic feature of the later "white-rot" stage is not a delignification of the woody elements, as was formerly supposed, but the removal of the thick lining layer from the walls of the wood fibres.

10. This internal layer of the wood fibre wall is rich in cellulose, but devoid of the aldehydes which usually accompany lignin.

11. The last walls of the wood elements to be attacked by the fungus are those of the medullary-ray cells.

12. Healthy Pershore and Victoria plum trees have been inoculated, using both spore and mycelial inocula. The results of the inoculation experiments indicate that *F. pomaceus* is only feebly parasitic on plum trees, the variety Pershore being slightly more susceptible than the variety Victoria.

This investigation was suggested by and conducted under the direction of Mr F. T. Brooks, to whom I should like to express my gratitude for advice and help.

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EXPERIMENTS ON FINGER-AND-TOE DISEASE (*PLASMODIOPHORA BRASSICAE*)

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The work here described was conducted at Newcastle-on-Tyne and Halle-an-der-Saale, in the Departments of Prof. M. C. Potter and the late Prof. G. Klebs respectively, from both of whom valuable help was received.

Part of the article was read before the British Association at their meeting in Johannesburg in 1905 and appeared in abstract in their *Report* for that year, but, owing to a revival of interest in the disease and a request for further details of the experiments, it seems opportune to publish a fuller account.

Most of the experiments were performed in pots, though for a few of them small plots were used. With pots, the material used was carefully mixed before potting; and in the plots, the soil, to a depth of about nine inches, of the whole area to be covered by an experiment was dug up, thrown into a heap, mixed and put back. Similarly, the infective material was carefully crushed (or chopped) and mixed before being used for inoculation.

In the earlier experiments the turnip, on account of its importance as a farm crop, was the plant chiefly grown, but experience led to its replacement by mustard, and to duplicating this with candytuft, which is much less liable to attacks by aphides and flea beetle.

Owing to their rapid growth, several successive crops of mustard and candytuft can be grown in a single season, and there is thus an opportunity of immediately repeating experiments. Turnips, on the other hand, require a whole season to mature. Mustard and candytuft have the further advantage of requiring very little space, so that larger numbers can be grown to maturity on a small area without serious overcrowding.

Species attacked

The disease has been recorded in most of the genera of Cruciferae. Judging by the proportion of plants attacked, I found the following very susceptible to the disease: candytuft, mustard, *Sisymbrium*, wallflower, and the numerous cultivated species of *Brassica*.

In an experiment at Halle on ground on which the disease had not been known to occur, spores from diseased swedes and turnips produced the disease in *Isatis* (5 attacked out of 20), *Cochlearia*(10 out

of 40) and *Sisymbrium* (27 out of 30), but not in *Draba*, *Erysimum*, *Hesperis*, *Matthiola*, or *Raphanus*, although the disease has been recorded for all these genera. Halsted (1896) had a somewhat similar experience with *Matthiola*. More recent work on the susceptibility of various Cruciferous plants to attack by *P. Brassicae* will be found in the publications of Gleisberg (1923), Gibbs (1932) and Rochlin (1933).

The disease has not been reported outside the Cruciferae, and attempts were made to infect certain plants nearly related to that family, or those having a similar ash composition (Wolff, 1871), but with negative results. The following were the species tested: *Reseda odorata*, *Corydalis glauca*, *Fumaria officinalis*, *Allium schoenoprasum*, *Urtica pilulifera*, and *Spinacia oleracea*.

The injury done by *Plasmodiophora* to its host

The extent to which attacked plants suffer from the disease varies with the species, but depends also on the age of the plant when it first becomes infected. Plants, of whatever species, when attacked late in life suffer comparatively little direct injury; but if attacked while still young certain species suffer severely, and either succumb or remain much stunted, whilst other species are apparently not injured by the disease. Turnips, charlock, *Sisymbrium* and wallflower suffer severely, and plants attacked when young show obvious symptoms of general ill-health apart from the tumours. Mustard, candytuft, garden cress and horse-radish, on the other hand, are apparently uninjured in general health, and the presence of disease cannot be detected without examining the roots.

The wallflower when attacked young, usually succumbs speedily, but if it survives, the seat of attack does not become a tumour but dries and splits.

If a section be cut through the root of a young diseased turnip it will be found that the disturbance of the vessels is very great, pieces being forced from the vertical into oblique and even horizontal positions (Kühn, 1858; Wakker, 1892). A detailed examination leaves the impression that the continuity of the vessels is broken to such an extent as to interfere seriously with their water-conducting function. This, no doubt, causes the well-known symptom of wilting of the leaves in turnips.

When turnips are attacked young the centre of disease is near the vessels which are disturbed by the cell multiplication and enlargement induced by the parasite. On the other hand, when infection is delayed, the seat of the tumour is superficial, and so far removed from the vessels that they are not seriously disturbed. Hence,

turnips attacked late in life show comparatively little indication of general ill-health.

A microscopic examination of diseased roots of two species that apparently are not directly injured by attack, showed that the vessels are situated in a hard fibrous strand, running down the centre of the root, and are not disturbed by the cell multiplication and enlargement caused by the parasite, which is confined to the soft, parenchymatous tissue surrounding this woody strand. There is, therefore, little or no interference with the ascending stream of water.

The occasional formation of tumours near the surface of turnips which already show considerable secondary thickening suggests that the parasite can enter the root long after the root-hairs have been shed.

Reaction of the soil

Three pots were filled with infected soil of practically neutral reaction; to one 5 per cent. of calcium carbonate was added (to keep it neutral), to another sodium carbonate, and to the third sulphuric acid. The two last pots rapidly lost their pronounced reaction, and the dressing had to be repeated frequently throughout the season. Of sodium carbonate, 0·2 per cent. was mixed with the surface soil at sowing, and smaller amounts were added throughout the season as tests indicated the alkalinity was falling. The reaction of the sulphuric acid pot was maintained by watering twice weekly with a 0·1 per cent. solution. Turnips were sown, and by the end of the summer the results were as shown in Table I.

Table I

	Total plants	Number diseased
Sodium carbonate	18	0
Calcium carbonate	13	4
Sulphuric acid	12	12

In all experiments on soil reaction, the tumours were examined microscopically to make certain that they were caused by *Plasmodiophora*. Within five weeks of sowing a duplicate experiment was started with an additional pot, kept acid with phosphoric acid. This experiment (Table II) was also conducted with turnips, and the reactions were maintained as before.

Table II

	Total plants	Number diseased
Sodium carbonate	7	1
Calcium carbonate	7	3
Sulphuric acid	7	7
Phosphoric acid	7	7

Owing to my absence towards the end of the experiment, the dressings were not continued until the end of the growing season; this probably explains the occurrence of some disease in the sodium carbonate pot, as, when the last dressings were applied, none of the plants in this pot appeared diseased.

The following duplicate experiment, also with turnips and in pots, was conducted the next season:

Table III

	Total plants	Number diseased	Total plants	Number diseased
Control—no dressing	8	4	8	3
Sulphuric acid	10	10	*0	*0
Phosphoric acid	10	7	6	5
Acetic acid	10	9	Omitted	Omitted
Quicklime	10	0	5	0
Caustic potash	10	0	6	0
Sodium carbonate	10	0	6	0

* Killed by strong acid in mistake.

In these experiments the reactions were maintained by watering thrice weekly with 0·3 per cent. solutions (saturated in the case of limewater) of the respective dressing. It will be noticed that acetic acid has the same encouraging effect as sulphuric and phosphoric acids, and that caustic potash and quicklime check the disease as effectively as sodium carbonate. We may therefore conclude that acidity is favourable and alkalinity unfavourable to the development of *Plasmodiophora*.

These experiments confirm those of Massee (1895) and Halsted (1896). They also explain the encouraging effects of dissolved manures containing an excess of acid; and the preventive action of wood ashes (Berkeley, 1856; le H., 1879). In Switzerland, in recent years, sodium carbonate has been recommended for application to infected soil to prevent finger-and-toe (Osterwalder, 1929), whilst in the United States, Chupp (1928), has shown that the disease increases very rapidly with increasing soil acidity.

In the following experiment, carried out with turnips in pots, the dressings, at the rate per acre stated, were mixed with the surface soil in spring just before sowing.

Table IV

Dressing	Total plants	Number diseased
Quicklime (2½ tons)	7	5
,, (5 tons)	8	1
Calcium carbonate (2½ tons)	8	5
,, (5 tons)	8	7
Calcium sulphate (1 ton)	8	7
,, (2 tons)	7	6
Potassium sulphate (½ ton)	7	6
,, (1 ton)	7	7
Untreated	7	5

As regards the action of quicklime, the number of diseased plants suggests that two and a half tons had done no good, whereas five tons almost entirely prevented the disease. There is, however, another important fact to record, namely, that until early in August the plants in both quicklime pots were all healthy, whereas at this date all the other pots, particularly the four receiving sulphates, had several diseased plants. Later in the season, the majority of the plants in the pot receiving quicklime at the two and a half ton's rate were attacked.

The following experiment, conducted with mustard, shows that the disease can be entirely prevented by quicklime, provided sufficiently large dressings are applied to the growing crop and frequently repeated.

Table V

Quicklime applied (rate per acre)	Total plants	Number diseased
Pot 1. Control (no dressing)	25	18
2. 5 cwt. repeated six times	20	6
3. 10 ,, repeated six times	24	2
4. 20 ,, repeated six times	23	0
5. 20 ,, repeated thrice	15	0
6. 40 ,, repeated thrice	33	0

The dressings in pots 2, 3 and 4 were applied and mixed with the surface soil on May 9th (immediately before sowing), June 1st and 14th, July 1st and 14th, and August 1st; and in pots 5 and 6 on May 9th, June 14th and July 14th. As will be seen from the table, they all materially reduced the disease, though only the last three dressings were sufficient to prevent it entirely.

By means of alkaline dressings, therefore, it is possible to delay infection, and if the alkalinity be maintained by repeating the dressings, the disease can be prevented. The frequent though delayed infection appearing in these experiments in pots dressed with quicklime allows one to infer that this substance, when applied to farm crops during or just before the growing season, acts in this way. It does not kill the fungus but merely keeps it in check, and as soon as the alkalinity falls the fungus again becomes active. Bremer (1924) also found that lime does not kill the spores.

In these experiments it was noticed that the degree of alkalinity required to hold *Plasmodiophora* in check was not detrimental to the host—indeed, the plants seemed to thrive better in slight alkalinity.

Acids encourage the disease; under acid conditions, plants are attacked early and turnips usually die off. Alkaline dressings, even when not repeated, delay the date of attack, and there is much less injury to the host. Thus, in one experiment (Table I), the turnips in the sulphuric acid pot, which were sown in May, were attacked very early and were all dead by the first week in August; whilst of the ten

plants, all diseased, in the pot dressed with the same acid (Table III), only two were alive at the end of the season. The delay in infection due to sodium carbonate (Table II) and to quicklime (Table IV) has already been referred to. There is thus a relation between the reaction of the dressing, the date of attack, and the amount of direct injury suffered by the host.

Under the influence of alkaline dressings infection is at least delayed, and the plants, even if diseased, are usually alive at the end of the season, whereas with acid dressings many of them die off early. Hence, in comparing the effect of preventive dressings, figures based on the number of diseased plants at the end of the season are apt to be misleading. Jamieson (1881) and Middleton (1902) have called attention to this in connection with field experiments.

Can the disease occur in soils rich in calcium?

That soils deficient in calcium are very subject to the disease has been well established by numerous soil analyses (Johnston, 1849; Voelcker, A., 1859; Voelcker, J. A., 1894; Gilchrist, 1896; etc.). To test whether richness in calcium confers immunity, a series of pot experiments with mixtures rich in calcium, and a series of analyses of soils expected to be rich in lime, were performed.

The pots were in duplicate and those of Series 1 (Table VI) were filled with mixtures of chalk and soil, the percentages stated being dry (moisture-free) chalk and dry soil. The chalk used was from the Chalk Formation, and analysis showed it to be practically pure calcium carbonate. Tumours grown the previous season were used for infective material and turnips were sown. Series 2 was prepared in exactly the same way, except that horse dung (litter-free) was mixed with the soil in each pot.

Table VI

	Series 1		Series 2 (Horse dung added to each pot)	
	Total plants	Number diseased	Total plants	Number diseased
100 % chalk	8	0	7	5
70 % chalk+soil	7	0	7	4
30 % ,,	8	0	7	7
10 % ,,	11	2	7	6
5 % ,,	7	5	8	7
2 % ,,	8	5	8	8

The table shows that the disease can occur in a highly calcareous soil and confirms the experiments of Potter (1896). The only pots in which the disease did not appear were three of Series 1, but in the light of subsequent experiments, the dryness of the soil here was

sufficient to account for the absence of disease. The object of adding horse dung in Series 2, and its effects, are discussed on p. 122.

Soil analyses

Each of the two gardens attached to the Agricultural Department of Armstrong College had a series of ten plots which, in 1895, were infected with diseased soil and had since been under experiment for the eradication of the disease. The two sets were duplicates, and a full account of their treatment and the results are given by Somerville (1894, 1895). These plots were selected for analysis because they were carrying affected crops and yet, as far as could be judged by rough tests, were rich in lime.

The soil of each plot was sampled in three places to a depth of one foot, and all estimations were made in duplicate. The results are given in Table VII. "Stones" refers to the portion which would not pass through a sieve with four meshes per linear inch, "Fine Soil" being what did pass through. The percentages of stones are given to make it possible to compare the figures with results which are expressed on the whole soil. The soil, as distinguished from the subsoil, was about one foot deep, and the physical character varied considerably.

Table VII

Plot	Treatment tons per acre	Percentage of stones	Percentage of total calcium in fine soil, calculated as CaO	Percentage of total calcium in fine soil, calculated as $CaCO_3$	Percentage of available calcium in fine soil, calculated as $CaCO_3$
	Applied in 1896				
1	1 ton lime	7·93 (7·28)*	2·38 (1·40)*	4·25 (2·50)*	1·71 (0·48)*
2	2½ tons lime	6·03 (6·10)	2·13 (1·77)	3·80 (3·16)	1·55 (0·92)
3	5 tons lime	5·68 (7·05)	1·97 (1·76)	3·51 (3·14)	2·63 (1·29)
4	Bleaching powder	5·76 (7·25)	3·02 (1·75)	5·39 (3·12)	1·35 (2·18)
5	Copper sulphate	9·16 (5·10)	1·79 (1·58)	3·19 (2·82)	0·74 (0·61)
	Applied in 1898				
6	Untreated	8·94 (7·27)	1·75 (2·59)	3·12 (4·62)	0·87 (2·39)
7	1 ton lime	6·42 (11·01)	2·19 (1·57)	3·91 (2·80)	1·50 (0·95)
8	2½ tons lime	8·75 (7·93)	2·13 (2·69)	3·80 (4·80)	1·35 (2·40)
9	5 tons lime	7·25 (7·85)	3·65 (1·53)	6·51 (2·73)	3·67 (1·09)
10	Copper sulphate	5·51 (7·11)	2·04 (1·42)	3·64 (2·53)	1·42 (0·61)

* The figures within brackets refer to the South Garden; the others to the North Garden.

The analyses show that the soil of the plots is very rich in calcium. Thus, calculating as carbonate, the lowest percentage is 2½, four plots contain between this and 3 per cent., ten between 3 and 4 per cent., and five above 4 per cent.; one contains even as much as 6½ per cent. A high calcium content need not, therefore, be a safeguard against the disease.

The amount of calcium present in an available form was also estimated. This was done by determining volumetrically the carbon dioxide expelled by dilute hydrochloric acid and calculating it to its equivalent of the carbonate. Even with calcium carbonate as thus determined the plots are all abundantly provided; the lowest contains nearly $\frac{1}{2}$ per cent., six contain between this and 1 per cent., eight between 1 and 2 per cent., four between 2 and 3 per cent., and one even contains over $3\frac{1}{2}$ per cent. Richness of a soil, even in calcium carbonate, therefore, does not necessarily confer immunity.

That the disease can occur in highly calcareous soil, and yet that soils naturally rich in calcium are much less subject to the disease than those deficient in it, must both, therefore, be accepted as established.

It has already been shown that lime can prevent the disease by making the soil alkaline, but it could do this only if applied to the growing crop or shortly before sowing.

But lime can also prevent the disease in another way, for which it needs time—often several years. The precise nature of its preventive action here is not yet known, but the cumulative evidence of farm practice and of numerous field experiments leaves no doubt as to the fact of its efficacy (Gilchrist, 1905). From the slow nature of its action it may reasonably be concluded that it is very indirect.

Action of neutral salts

To test the action of neutral salts on the disease, the neutral phosphates, sulphates and nitrates of potassium, sodium and calcium, were applied to pots of infected soil. The experiment was carried out in duplicate. In the mustard series (Table VIII), the dressings, at the rate of a ton per acre, were mixed with the soil at potting time, while in the candytuft series they were applied in water (0·2 per cent.) twice weekly.

Table VIII

	Mustard		Candytuft	
	Total plants	Percentage diseased	Total plants	Percentage diseased
Control (no dressing)	40	39	47	30
Neutral potassium phosphate	47	43	60	36
Potassium sulphate	46	39	50	50
Potassium nitrate	39	8	70	14
Neutral sodium phosphate	33	33	47	27
Sodium sulphate	36	29	54	44
Sodium nitrate	45	7	53	19
Dicalcic phosphate	40	55	79	26
Calcium sulphate	35	30	72	30
Calcium nitrate	35	11	65	12

All three nitrates, in both series, markedly reduced the disease. In the mustard series they gave an average of under 9 per cent.

diseased, whereas the control pot contained 39 per cent., and the other six from 29 to 55 per cent. In the candytuft series the nitrate pots averaged 15 per cent. against 30 per cent. in the control, and from 26 to 50 per cent. in the others. This experiment was repeated the following season, again in pots and in duplicate, the soil being sandy with some humus. The results were very similar except that there was less disease in the undressed pots.

In the experiment shown in Table IV sulphates of both calcium and potassium caused the disease to appear earlier than in any of the other pots, and the attacked plants in the sulphate pot were visibly dying of the disease early in the season. The earlier appearance of disease is evidence that sulphates encourage the fungus, but the total number of turnips grown in the pots is too small to admit of accurate comparisons based on numbers. The soil was rich in organic matter.

The encouraging effects of sulphates were not noticed in the experiment with mustard and candytuft described in Table VIII. Earlier infection is not excluded, however, as mustard and candytuft show no symptoms of infection apart from the tumours which are usually hidden in the ground, whereas in turnips early infection is evident from the wilting of the leaves.

Thus in one experiment nitrates reduced the disease and in another sulphates encouraged it, but the principle underlying the action of neutral salts is not apparent from these experiments.

An effect of organic matter

Some years previous to this work, Prof. Potter observed that a piece of ground to which he had applied a watery extract of horse dung produced the disease much more virulently than it had ever done before. This observation was followed up by several experiments, in which the pots were of burnt clay, the horse dung was the solid excreta, free from litter, and the sand pure washed silver sand, containing less than 0·1 per cent. available organic matter. Where sand was used without dung, a small amount of mineral matter was added to supply the elements essential for growth. Crushed turnip tumours were used for inoculation.

In two pots, one of silver sand and the other of the same material mixed with horse dung, both artificially infected, turnips were grown, with the following result:

	Total plants	Number diseased
Pure sand, plus essential minerals	20	0
Pure sand, plus horse dung	12	10

The results suggest that organic matter is essential for the occurrence of the disease, and this seems to be confirmed by the Chalk

Experiment (Table VI, p. 119), which was carried out the same season. The chief object of this experiment was to ascertain whether it is possible for the disease to occur in a highly calcareous soil, but a duplicate series of pots was added, in each of which a heavy dressing of horse dung was mixed with the soil. In pots containing 30 per cent. or more of chalk the disease occurred only where horse dung was added; and in those containing less than 30 per cent. chalk the addition of horse dung increased the amount of disease.

Tests of the reaction of the pots of the Chalk Experiment throughout the season showed that the horse dung increased the alkalinity, so that the dung was evidently not favouring the disease by making the soil acid.

Potter's observation was based on the use of a watery extract of dung, and the only other likely explanation of its action is that organic matter is needed as food for the fungus—in other words, that *Plasmodiophora* requires to lead a saprophytic existence in the soil before entering its host. This would be extremely important, and it was therefore decided to repeat the sand experiment.

The experiment was performed in duplicate with mustard and candytuft, with the following result:

	Mustard		Candytuft	
	Total plants	Number diseased	Total plants	Number diseased
Pure sand with essential mineral (inoculated)	30	19	51	11
Pure sand and horse dung (inoculated)	40	24	135	50
Sand and horse dung (control—not inoculated)	60	0	—	—

The experiment showed that the disease could occur in the absence of organic matter.

The problem now was to reconcile this result with that of the previous season. Apparently the chief difference which could be of importance between the two sets of sand pots lay in the amount of water given. Those of the first year had been watered only once a day, whereas in the following season they had been watered twice daily. As a consequence, the pure sand pot in the former was often dry, while in the latter the sand remained comparatively moist. On this interpretation the disease should occur in soils devoid of organic matter provided the soil be kept sufficiently moist. This was tested in various ways. In one experiment two pots of the same sand as in the last experiment were taken. Pot A was kept continuously wet throughout by standing it in a bowl of water which was never allowed to go dry, while pot B was kept comparatively dry by giving it free drainage and watering only once daily. At the end of the season pot A had seventeen plants, of which nine were diseased, and

pot B ten plants, all unaffected. In a duplicate, conducted in the same sand, pot A had seven diseased plants out of a total of thirty, but pot B was kept so dry that the plants died prematurely.

Another medium devoid of organic matter in which the disease occurred, provided it was kept moist, was one consisting of the same kind of sand mixed with kaolin.

On the other hand, it was shown that the disease could be prevented in a soil rich in organic matter by keeping the soil dry. Thus, in a mixture of sand and horse dung (which was known from other experiments to be suitable to the disease) with which this precaution was taken, only two plants out of thirty were diseased; and in a duplicate pot, containing fourteen plants, all remained healthy.

The retention of moisture, then, was the explanation of the influence of horse dung in my experiments, though this interpretation is not applicable to the case observed by Potter.

The disease has been recorded in water cultures, in which there would presumably be no appreciable amount of organic matter.

In the initial stages of these experiments on organic matter, well-burnt ashes were employed in one experiment as a medium of growth. Analysis showed them to be suitable for this purpose as far as the available organic matter was concerned, but no disease occurred either in the ashes alone or in the pot containing ashes mixed with horse dung. In the light of other experiments then in progress, the pronounced alkalinity of the ashes was a sufficient explanation of the absence of disease.

Germination of the spores

Periodically during two years attempts were made to induce the spores to germinate. The attempts were made at all seasons, in a great variety of substances and generally under very diverse conditions, but always with negative results. Meyer (1888) and Frank (1896, 1897) record a similar experience, though Woronin (1878), Rostrup (1885) and Chupp (1917) report having observed the germination.

In two successive seasons, towards the end of May, the majority of the spores in some diseased roots produced the previous year and lying in a heap out of doors were found to shrivel and die. There was no evidence that they had germinated, and attempts to observe the germination of the few unaltered spores also failed. Meyer (1888) records a similar experience.

Are the spores capable of immediately infecting another plant?

In July, diseased turnip tumours grown that season were washed to remove anything that might be adhering to their surface and used to infect pots, the soil in which was free from disease but known from other experiments to develop the disease after infection. The pots were sown on July 20th with turnips, candytuft and mustard. On October 15th, when the plants were harvested, they were all free from disease. The results suggest that the spores are not able to infect another host in the same season.

Soil at Halle that had borne badly diseased plants that season was sown with mustard on November 14th. When the plants were taken up on March 15th following, none were diseased. This suggests that infection does not occur during winter.

To what depth in soil can the spores infect plants?

Infective material was sown on the floor of trenches two, four, six, eight, ten and twelve inches deep, care being taken that none came in contact with the sides of the trenches, and these were afterwards filled up level and sown with turnips. Plants were attacked in all the trenches, but it was found that in the deeper trenches the tumours were confined to the approximate depth at which the spores were sown. This was particularly noticeable in the twelve-inch trench, and suggests that the parasite settles in the host near its point of entrance.

The soil was light and porous in character, and for some years previously had been cropped with lucerne, the roots of which no doubt kept it open. This experiment was on the same lines as that of Potter (1896), where in strong clay the disease occurred to a depth of only six inches.

General summary

1. Attempts to infect with *Plasmodiophora*, plants allied to Cruciferae taxonomically, or with a similar ash composition, gave negative results.

2. The injury done by *Plasmodiophora* to its host depends on the species of the host and on the age at infection. Some species are apparently uninjured. Where considerable direct injury was suffered the continuity of the vessels was found to be broken.

3. Acidity is favourable and alkalinity unfavourable to the development of the disease. By means of alkaline dressings infection can be delayed or entirely prevented. Quicklime when applied to the

growing crop inhibits the disease by its alkalising action. Lime can also prevent the disease in some other, as yet unknown, manner, for which it needs considerable time.

4. The disease can occur in highly calcareous soils and yet soils naturally rich in calcium are much less subject to the disease than those deficient in it.

5. The application of sulphate to soil encourages the fungus as judged by the earlier appearance of the disease.

6. A moist soil is favourable to the development of the disease.

7. The presence of organic matter in soil is not necessary for the development of finger-and-toe, though it may encourage the disease by enabling the soil to retain its moisture.

8. Attempts to observe the germination of the spores failed. Experiments suggest that the spores are not able to infect another host in the same season and that infection does not occur during winter.

9. The spores were found to infect plants to a depth of twelve inches in soil.

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L. G. WINDT AND HETEROECISM

By J. Ramsbottom

The history of the discovery of heteroecism is of considerable interest. There are several accounts of the early belief that the presence of barberry is harmful to corn but few of them refer to L. G. Windt's *Der Berberitzenstrauch, ein Feind des Wintergetreides*, published in 1806; indeed the only mention in readily accessible works is in Klebahn's *Die wirtswechselnden Rostpilze*, pp. 210–13 (1904). Windt's book is apparently very rare, for the only copy I can trace in this country is in the British Museum. However, in the Department of Botany there is a manuscript translation which was formerly in the possession of Sir Joseph Banks. It was written in Banks's library but I cannot recognise the handwriting*. The title is given as "The Barberry-bush an enemy to winter corn, proved by observations, experiments and testimonies by L. G. Windt, counsellour in the chamber of accounts of the Count De Lippe Schaumberg."

It is not an easy matter to assign the proper amount of credit due to individuals for the part they played in the controversies that took place on the subject of heteroecism at the beginning of last century. Too much stress is likely to be given to those points which in later days were proved, with the omission of others which were wide of the mark. Bearing this in mind, however, I feel that Windt has not received his due share of the credit. This may have been because of the scarcity of his book, but no doubt its haphazard arrangement counted against the acceptance of the evidence brought forward; it might almost be said that the only orderly part of the book is the preface. "The honoured reader will easily perceive that the observations and transactions, which I here lay before him, were not, with the exception of a few extracts from newspapers and journals, originally intended for the press.

"In the year 1804, I was led into an examination. . . whether Barberry bushes were hurtfull to wheat and rye, growing in their neighbourhood. I had probable grounds of suspicion, and carried on my inquiries with all the attention that other avocations and duties would permit, though solely with a view to the benefit of my prince and the advantage of that part of my fellow subjects employed in agriculture. But when the hurtfullness of the Barberry appeared to be fully verified,

* "Not carrying with me the German text to Sir Joseph's library I had added the erazed most important paragraph." MSS. p. 102.

Errors in the spelling of names of both fungi and botanists have been corrected in the extracts quoted.

I felt myself compelled by duty to make a communication of this fact to the *Imperial Intelligencer** and other public prints, that, after benefiting by the notices and advice of learned and experienced husbandmen, I might bring the matter, more confidently, before the Publick. I knew not, as yet, the bad reputation of the Barberry in Holland, England, and even in America. My acquaintance with books on rural economy was so scanty, that I had not heard of any discussions, or even hints, on this subject, particularly of what is said on it, by Mr Marshall, and Mr Begtrup....

"From the collections before me, the evil report of the Barberry will, I am convinced, appear to be by no means founded on vulgar prejudice: its destructive effects, talked of in many places, and still called in question, will be shown clearly to exist, and especially in the case of rye....

"Various occupations do not allow me to bestow more time on this work, than is necessary to explain, in an introduction, the disease in corn called rust, and to give some account of the Barberry, to whose operation this malady is ascribed: the remainder consists intirely of diaries, records, and testimonies; which documents I have obtained permission to publish, in consideration of their usefullness to the farmer. Had I been possessed of leisure and ability to reduce the materials of this work into a more regular composition, the Public would not probably be a gainer, since many internal marks of truth might thereby have been smoothed away, and effaced. Here, the whole rests on facts, which each reader will weigh for himself, without being anticipated in his judgement."

It is interesting to note the circularising of the Press which was practically contemporaneous with the effort made by A. Young, the Secretary to the Board of Agriculture in this country, to obtain information from farmers, landowners and others about the cause of mildew in wheat and the effect of barberry (*Annals of Agriculture*, XLIII (1805), 457).

Windt began with very mixed ideas about the nature of the disease in corn and the manner in which barberry was connected with it. He sums up by saying "it would ill become me to perplex the learned reader by stringing one hypothesis on another, in order to defend my own conjectures. The operation of the Barberry may be referred to the article of occult qualities: it is enough for the husbandman to know that it hurts his corn."

The diary and other documents "either wholly, or in abstracts" are given "according to the order of time in which they were collected."

The first memoir is dated July 8th, 1804. It records that the great garden at Hockersau was, above eight years ago, hedged with Bar-

* *Der Reichsanzeiger.*

berry, and that whenever sown with rye has suffered by mildew, and full details are given about neighbouring fields. The evidence is for the most part very clear: thus "the swathes, next to the hedge, looked like chopped straw: those farther off were less broken: but even in the divisions considerably remote, the rye was observed to look red or yellow." Windt's belief, however, was that barberry had a certain mischievous effect on wheat and at first he does not distinguish between rust and smut. Also he records without comment that "An enclosure had been made with this plant [barberry] round a field of Beans: the beans were ruined by mildew: the hedge was removed; and the evil ceased."

The memoir concludes: "When all these facts are considered, the mischievousness of this plant is rendered highly probable: yet the evidence falls short of certainty. Before condemning the Barberry to be rooted out, exact experiments should be made, so that every degree of doubt may be removed. This preliminary information I have laid before the Count's chamber, humbly wishing to be instructed whether the Barberry hedge should at all events be rooted out next harvest, and a different kind of hedge planted. If suspicions are confirmed by future experiments, it will be necessary for the benifit of agriculture, to root out the Barberry."

The memoir was not presented until August, by which time he had collected further evidence. He had become "so thoroughly convinced of the hurtfullness of the Barberry" that he proposed replacing it by hornbeam in certain named gardens. He submits certain proposals and says: "I thought it time, however, to render this account to the chamber, that, before the rye of this year is cut down, the facts, which I have stated, may be examined and verified by others at any rate, I think it should be ordered that private persons root out all Barberries from their grounds, under pain of punishment." In the appended notes he first describes an experiment of placing three boxes of rye plants, one on the north side of a barberry hedge almost touching the leaves, a second one hundred paces away and the third on the south side at an intermediate distance. The rye became infected, that on the south side less than the others. "I wished now to examine the field from which the boxes of rye had been taken; but the time for this had gone by." Observations were practically continuous—the account of the experiment is dated "morning of the 16th July 1804," the next observation "of the same date in the afternoon" and the following one "the same date—in the evening."

On August 18th he states that the rye in the field from which the boxes were taken "was mildewed, in the present as in the last year: the ears shrivelled or empty: a circumstance which leaves the effect of the Barberry doubtfull." Later he had "recourse to the only other mode, by which the hurtfullness of this plant could be proved."

Towards the end of October 1804 he caused "eight of the soundest Barberry bushes bearing fruit" to be transplanted. "Two of them were placed near the rye, 40 steps asunder. A third was planted by mistake among wheat. At the distance of 800 steps from these three, the five others were planted among rye, distant 50 steps, respectively, from each other. It was scarcely possible that an experiment made on so large a scale should not afford conviction. To me, who was convinced already, it seemed grievous to destroy much good grain, but the present loss, I hoped, would be over balanced by future advantage."

This experiment was reported on as follows: "Agreably to my received instructions, I planted a few Barberry bushes amidst the rye, in order to ascertain the effect: but did not discover, though frequent and curious in my visits, that the rye near to these decried bushes looked worse than any other. It seemed beautifull, even on the 27th of last month: but eight days later, that is, the 4th of the present month, I found, on examination, that it had begun to suffer from the dangerous neighbourhood. The evil has recently so much encreased, that the rye is now quite ruined, as the chamber will be convinced by the accompanying samples. This effect is the more striking, because the corn a little inward continues perfectly sound.

Maschvorwerk 8th July 1805
Stahlhuth."

Windt proceeds: "The chamber of accounts and revenue does not in the least distrust the report of the able and respectable superintenant Stahlhuth, yet wishes to have it confirmed in a formal and legal manner. For this reason, the Baillif of Buckeburg is commissioned to take the evidence of intelligent farmers on the subject, who are to answer the following questions. I. How is it with the rye, as to the stem, leaves, and ear, in places contiguous to Barberries, compared with others more remote from them. Are there signs of disease? II. How is it with the Barberries themselves. Are they green and luxuriant, or decayed and weakly, with signs of malady on their leaves and branches? III. What is the state of the rye near to these bushes? Are the stalks and blades spotted? Are the ears well filled, or empty? Is the colour sickly? and what is the name of the malady with which they are attacked? IV. How many feet inward in the field, does the mischief extend? Is it always worse, the nearer you come to the Barberry. V. In other parts of the land, are there any signs of a similar malady?

"This commission was carefully fulfilled by the Bailif of Buckeburg."

Four farmers examined the field and their evidence is given. Summarising this: The rye was affected to from ten to fifteen paces round the bushes, and not elsewhere. The leaves of the Barberry bore "small

tubercles" in "dry spots." Two of the witnesses stated that rye was always diseased where barberry grew, that the disease was more severe near the barberry and chiefly in the direction of the wind. Windt himself visited the field on July 20th and found that by then the rust had extended to two hundred paces. So early as July 20th, 1804, Windt writes: "The thing, that I learned today, of most importance to my enquiry, was the following. I walked along the hedge enclosing H.——'s field, between the hedge and the rye. The shepherd's son had said, that here no barberries were to be found. I therefore passed forward, careless of the hedge, and looking only at the corn. At once I saw before me, rye in as bad a condition as at Hockersau close to the Barberry hedge; white, dry, the ears shrivelled, and the stems broken. It lay in a half circle of six paces in semi-diameter, from the main stock of the hedge. It seemed, as if it had been beat down with a club. This appearance, which very much surprised me, turned my attention to the hedge, where I immediately discovered a Barberry bush to be the source of the evil. I advanced straight forward amid the corn, and found, even where the stems were upright, the ears quite shrivelled; and the mischief extending deeply into the field. This striking observation, concurred with what is before said of H.——'s field, where the mildew in the rye encreased with its vicinity to the Barberry, to assure me that this plant, at least in our climate, has the most pernicious effects. As far as the conviction of my own mind is concerned, I have no more inquiries to make." He, however, goes on accumulating evidence: "After such unequivocal proofs of the hurtfullness of the Barberry, two questions naturally arise, over what extent of country does its operation prevail; and secondly, does the Barberry, unaccompanied by circumstances, produce the mischief; or must not its hurtfull operation be aided by a certain disposition of the lower atmosphere." Regarding the distance these distinctive effects extend he says: "all my enquiries and observations show that these effects are striking and desolating in the distance of 10 or 12 paces. I have also perceived them visibly at the distance of 50, 100, 150 paces:" and a final attack "above 1000 paces."

"In the near neighbourhood of the Barberry, the rye is attacked before, or during the bloom: the rye farther off, I observed to be damaged later, yet totally; that still more distant, as the Horst field, yet later, and in a smaller degree, so that some produce might be expected from it: the remote fields Petzerhofe and Gevallernfelde, were injured the latest of all; from these observations, I conclude that the infection diffuses itself on all sides as from a center, and thus the extent of the mischief is very great."

Then follows the evidence of four farmers one of whom says: "In Eisbergen, the Barberry is called the air-bush, from a notion that it attracts the air. This plant grows in two places of a hedge there; the

rye is always rusty near those places, as may be seen remarkably in the present year."

The evidence up to July 20th was transmitted by Windt "to those concerned, and by them sent for the examination and opinion of a skillfull Botanist. This opinion was, that though the mischief from the Barberry seemed highly probable, yet, before the extirpation of so usefull a plant, more observations ought to be made, more experiments to be tried, and information obtained from different places, whether in them also, the Barberry was found to be hurtfull. Accordingly, I received instructions in the beginning of October. I. To root out the hedge 1,500 feet long round the great garden at Hockersau, and to plant another in its stead. II. To continue my observations and experiments. III. To make inquiry through the public prints, whether the mischievousness of the Barberry was elsewhere experienced."

Windt then sought information from a farmer who had Barberries in a hedge "contiguous to the tilled land of peasants there. The peasants imagined that their crops were hurt by the hedge, and entreated him to remove it. He hesitated, and endeavoured to reason the peasants out of their opinion. These continued to murmur, untill a compromise was entered into, by which the Barberries were removed, and a thorn hedge, at the expense of the complainants, planted in its stead. When this was done, the corn continued to suffer by mildew, as much as formerly."

The farmer answered all his questions, holding that "the whole parish saw that the expence of removing the hedge was thrown away." Windt's only comment is: "Mr Meyer reasons like an observing intelligent farmer. As the mildew happened, notwithstanding that the Barberries were removed, he could not regard them as its cause."

He reported to the chamber of revenue that he had "rooted out the hedge round the great garden of Hockersau (which was done in the first days of the month) and filled, with the useless bushes, a pond in the neighbouring meadow." To this I added, "That there were many Barberries mixed with other shrubs in the Bosquet of Klus, contiguous to the fields of Hockersau. I had not yet ascertained how far the mischief from Barberries extended; but had reason to suspect that it spread very widely, in consequence of the contagion diffused from one corn field to another. In the neighbourhood of such fields, I thought that Barberries ought not to be tolerated even in woods and thickets, because the infection from them might be conveyed by the air, and because they might, through inattention, be transplanted, and again employed as hedges. It appeared therefore highly proper, that they should be extirpated not only from the above mentioned Bosquet, but from the possessions of the peasants and others in the district of Hockersau and the adjacent villages. If this should be followed by

happy effects on the quality and quantity of the grain, the very extensive mischief from the Barberries would be clearly established. The Count's chamber, duly attentive to the interests of agriculture, determined, accordingly, that this opinion should be made known, and strongly impressed by the magistrates: that I should proceed with all diligence in my observations and inquiries; and, if these afforded the result expected by me, that the Barberries, a plant that might be well dispersed with, should be extirpated through out the whole country. According to these instructions, my conduct was regulated. The peasants, under the direction of a forester, laboured with alacrity in clearing the Bosquet of Klus the 18th of March: and in employing the rooted out bushes, which had been piled up in an open field, to fill pits in the neighbouring meadow. In other parts of the country, the magistrates were contented to injoin, but the Baillif of Buckeburg commanded the extirpation under pain of punishment. A few years will determine, whether the mildew may be prevented, that has impoverished so many substantial farmers, and which beggared many in 1805."

The next observation deals with a disease of sheep thought to be in some way associated with rust and "Barberries at least cooperate."

He next mentions a field with two barberry bushes in each of two hedges: the rye was then healthy, but he asks that the field should be examined later and forecasts what would be seen. They were examined on May 25th and found to be free of rust. The same two observers again visited the field on July 25th and reported that where the barberry bushes grew the rye was rusted, the disease decreasing with the distance from the bushes. In another field no barberries grew "and here the rye was in the soundest and most healthy state possible."

On August 1st he examined some fields and found the corn in one totally ruined though more distant from the barberry and separated from it by two hedges by a causeway with trees on either side and by a deep stone quarry. "The difference, I think, arises from the wind, which, for a month past, has blown chiefly from the west, changing to the east seldom, and that only for a moment. The west wind conveyed the pernicious influence of the Barberries to the Black close: but the fields west of the Bosquet, naturally escaped the contagion. This observation seems conclusive; though the only one of the kind that it has been in my power to make. If the mischief done by the Barberries depends on the state of the wind, it is clear that their operation consists, not as many country-people imagine, in drawing the healthfull influence from the air, but, on the contrary, in exhaling something that is pernicious, whose effects appear in those fields to which the wind carries it."

He adds a note regarding the possibility of other plants being de-

leterious to corn and records that a twig of *Cornus sanguinea* was brought to him from a field which, although without barberries, had rye completely ruined by rust. The *Cornus* had diseased leaves "as the leaves of plum-trees are, when attacked with what is called the honey-dew."

He ends his own observations thus: "My remarks are now concluded, and I have only to mention what was done in consequence of them.

"When the Barberry bushes among the rye were removed, and the corn brought from the fields, I considered the business as ended. The hurtfullness of these bushes was now evinced by my own observations and experiments, by the communications and testimonies of others. The chamber of accounts, taking all this into serious consideration, declared that the question was decided as clearly as the nature of the thing admitted, or as practical purposes required. Accordingly, the matter was laid before government, and a general order issued. I. That all Barberry bushes should be rooted out from hedges, gardens, fields, or plantations near fields, before the 1st December 1805. II. That persons contravening this order should be fined two dollars. III. That the fines should be bestowed in rewards to informers. By these means, the Barberry has been extirpated from the district of Buckeburg, where it formerly abounded; or, if any lurks concealed here or in other parts of the country, it will certainly not be able to defend itself against the zeal and avidity of informers."

For the convenience of his readers he adds the principal articles that have appeared on the subject in the newspapers during the past year: others he summarises. As is to be expected, the correspondence is not one sided. Much additional evidence is given, however, of the effect of the barberry.

Although they do not concern Windt's own work it will not be out of place to consider the three most important articles.

The first is by Baron von Montelon. It is an account of the injury done to winter corn and imputed to the influence of barberries, a paper read before the Economical Society Brandenburgh, November 9th, 1804, and published in the *Imperial Intelligencer*, No. 26, January 28th, 1805. It gives a clear description of various observations from 1800 onwards. When describing the effect of some barberry bushes on rye he adds: "Comprehended between two sides of this lost rye, the north and east side, I had sown 30 bushels of wheat; this continued to be good. It yielded 338 shocks, from which were threshed 375 bushels* of fine sound wheat. The rye plants that happened to grow amidst this wheat, were as bad as the rest; whereas the wheat stalks that grew among the diseased rye, remained perfectly sound. This circumstance seems the more worthy of notice, because the English writers on husbandry, and particularly Mr Marshall, complain of the

* "He says 15 wispeln and 15 scheffel. A wispeln is 24 scheffel, or bushels."

mischief accruing from Barberries to wheat. The wheat here, was indeed 1,000 paces from the pestiferous bushes. Notwithstanding this distance, the rye was destroyed. A peasant sowed a single bushel of wheat, at a considerable distance from mine, but nigher the vineyard; this continued in the midst of the shrivelled rye."

Klebahn recognises this as an early record of specialisation.

Two other notes may be given in full.

The first is "Conjectures on the cause of the pernicious influence of the Barberry on rye occasioned by the communications in the Imperial Intelligencer, No. 36." "The Editor of those remarks wishing for the opinion of others concerning the cause of the pernicious influence of the Barberry, I venture to impart my thoughts on the subject, hoping for indulgence as I am neither a philosopher nor an agriculturist. I am perswaded that the black spots, like fly-bites on rye and barberry, are nothing else but small fungi or mushrooms. These grow on many other kinds of plants, witness different sorts of *Erysiphe*, *Aecidium*, *Puccinia*, *Uredo*, etc., and most commonly on the leaves. In my opinion the spots on Barberry are nothing but the *Erysiphe Berberis* (Hedw.) which usually grows on the leaves, and whose wonderfully small seeds, being carried towards corn fields by the wind, fix their roots and vegetate in the stems, depriving them of their juicy nutriment, and thereby rendering the ears small or empty. As these diminutive mushrooms attain puberty in a very short time, (which is the case with the *Mucor Mucedo* of Linn. and various other kinds) and the seeds are thus again shortly fit for the reproduction of new seeds, which are carried by the wind to neighbouring fields, it seems likely that one ear infects another, and when the wind continues constant for a considerable time, that the contagion may thus spread very widely. It would be of importance to examine by the microscope whether these small, spot-like, mushrooms on the Barberry, are of the same sort with those on rye. I greatly wish that some skillfull Botanist a Persoon, Bridel, Wildenow*, Roth, or Sprengel, would communicate their remarks on this subject in the *Imperial Intelligencer*. That the flower dust, or vapour from the plant, should extend to such distances, does not appear very probable.

E—s—n berg. 14th Feb. 1805."

The second is "Natural Knowledge. Influence of the Barberry on Corn, in reference to No. 188 of the *Imperial Intelligencer*."

"Where experience pronounces clearly, all doubt ought to cease.

* C. L. Wildenow (*Beitr. Naturh.* Fr. Weber und M. H. Mohl (1805), p. 132) from observation concluded that *Aecidium Berberidis* produced *Uredo linearis* on corn. He rubbed leaves of *Elymus*, *Populus balsamifera* and *Sorbus Aucuparia* with infected barberry leaves. He found a small area of rust on the poplar and stated that *Aecidium Berberidis*, *Uredo linearis* and *U. popularis* were forms of the same growth, the epidermis of the host plant determining the form of the parasite. The genera *Aecidium* and *Uredo*, therefore, should be sunk and he proposed the name *Ustilago pulvis* for the fungus to prevent confusion. He published no further account, though he had intended repeating his experiments the following spring.

That Barberries occasion rust and the failure of crops, is a conclusion forced on me by my frequent botanical excursions through this country. For this conclusion, I have now found the completest authority in a most interesting paper on the trust-worthy Sir Joseph Banks: 'A short account of the disease in corn, called by Farmers the Blight.' In the country near Rollesby in Norfolk, where Barberries abound, the wheat so seldom thrives that the place has acquired the name of mildew Rollesby.

"But the manner in which Barberries operate their mischievous effect, is not easily explained. The pollen or fecundating dust of their bloom is not calculated for such a purpose: this waxy substance is altogether harmless.

"It is known that Barberry-leaves are much infested with a fungus (*Gastromgius*) the *Aecidium Berberidis*, first delineated by Jacquin (*Collect.* vol. I, Tab. 4) and afterwards by Rebentisch (*Flor. Neomarch.* Tab. III, fig. 2). The rust in corn is commonly called *Uredo*, and held to be merely a powder which corrodes and cracks the Epidermis. But the more accurate observations of Persoon (*Dispos. Math. fung.* t. III, f. 3) and of Banks (*l.c.* Tab. I) prove it to be a *Puccinia*, called the *Puccinia graminis* by Persoon (*Synopsis fung.* p. 228). Magnificent, exquisite, incomparable are the delineations which Banks, with assistance of the King's botanical painter Francis Bauer*, has given of this production in the work above cited. I lament that this small work is not on sale, and only given by the author in presents; for the art with which these microscopic bodies are represented is worthy of the greatest admiration.

"We here see clearly how these fungi insinuate and root themselves in every fissure of the stalk or leaf, how they project from a small tubercle, are formed into a pear-like body, divided by a septum, finally how they burst at the upper end, and scatter their seeds. Banks reckons that every fissure (and there are 500 fissures within the 12th part of a square inch) contains from 20 to 40 of these mushrooms. He shows that the countless multitude of these mushrooms robs the corn of its nourishment, and prevents the developement of its grains. All this is most excellently explained by him.

"The mushrooms on the Barberry, and those called rust on corn, belong to the same family (*Gastromgii*) but are not of one genus. The *Aecidium* has cylindric seed-bags, which burst with notched openings. The *Puccinia* has top-shaped seed-bags, separated by partition-walls or septa. Is it possible for one genus to change into another? Not surely, in completely organised plants. An oak cannot become a beech; nor a pear-tree, an apple-tree. But, here, are most diminutive productions whose whole vegetable character is stamped at once in their formation. May not the seeds of *Aecidium Berberidis*, by trans-

* Francis Bauer's original coloured drawings of "Diseases in Corn" are in the Department of Botany, British Museum (Natural History).

plantation on grass or corn be converted into *Puccinia graminis*? I do not assert that this happens: I only ask the question.

"SPRENGEL, *Professor of Botany*."

The book ends with a summary of Banks's well-known letter: "I did not choose to conclude and publish this work, untill I had seen the account written by Sir Joseph Banks in 1804." Windt gives a very full summary of it and makes microscopical examination which leads him to the discovery that the rust spores in rye were egg-shaped, whereas those in wheat were round—a morphological difference of some importance. "I readily renounced my former opinion: my own eyes now convinced me that what I had taken for mere globules of sap, consisted of finely organised bodies. The passages in which I had proposed my erroneous hypothesis, it is too late to cancel. My main object, however, is not affected by this error, and I am happy to have come at the truth, tho' by a circuitous route. I leave it to philosophers to explain why the rust scratched from stalks of rye, is egg-shaped, and that from wheat, spherical; and also to determine whether the same kind of mushrooms may not assume different forms when nourished by these two plants, through the difference in their sap." Klebahn points out that it is probable that the wheat was infected with *Puccinia triticina* or *P. glumarum*, not with *P. graminis*.

Windt is not fully satisfied that the spores from the rust on barberry can "take root on corn in whatever condition it may be, diseased or sound....Is the blight in corn caused merely by the vegetation of small parasitic fungi, or must not a chemical ferment concur, communicating from one plant to another, chillness, corruption and a stopping of the circulating juice?"

"I conclude this book in the perswasion that, the point, which I aimed at, is established....To those still inclined to regard the Barberry as innocuous...I would only make the request, that these no longer urge their opinion on abstract and general grounds, untill they have collected the result of impartial observation and carefull experiment. To be convinced by their own eyes let them plant Barberries among rye, and compare their effect with that of hazel or horn-beam: towards the end of June let them cut off a few Barberry-branches with their leaves, some of which branches are infected with rust, and others in a sound state. Let them be respectively scattered in different places among the corn, and the consequences carefully remarked. Justice requires, not only that such experiments should, by every man, be confined to his own possessions, but that, for the safety of his neighbours, the deleterious vegetables should be destroyed, as soon as their operation has been ascertained." He aimed at the extirpation of the Barberry "so completely, that no mischievous vestige of it shall remain in our fields" so that in a few years in the northern parts of Germany it would be "as rare an object as the palm-tree of the East."

A DISEASE OF POMEGRANATE (*PUNICA GRANATUM* LINN.) DUE TO *AMPHICHAETA PUNICAE* N.SP.

By H. CHAUDHURI and JAGTAR SINGH

(*From the Department of Botany, Panjab University, Lahore*)

(With Plate II)

The disease was noticed on the nursery plants in Lawrence Garden, Lahore. The twigs of the infected plants bore innumerable minute fruit bodies of *Amphichaeta* (Pl. II, fig. I). The disease was confined to the twigs. Infected plants were seldom killed, but they were much stunted in growth. Hand sections and microtome sections 8 μ thick were made. These showed hyphae mainly in the vessels of the secondary wood (Pl. II, fig. II), with some also in the cortex and in the pith.

The hyphae are branched, generally unseptate, and occasionally dark coloured. Acervuli are freely formed on the surface of the twigs as well as in culture. They are of various shapes and sizes and contain numerous conidiophores bearing conidia.

The fungus is placed in the genus *Amphichaeta* McAlp. for the following reasons. The spores are borne in acervuli (Pl. II, fig. VIII) with a stroma-like stratum without setae and are not borne in chains; they are dark, at least in part, and the conidia are uniciliate at each end. The spores are large, measuring 22–27 × 7–11 μ, spindle-shaped, and 5-septate, the four middle cells dark coloured, the terminal cells hyaline. Both the cilia are sharply curved usually on the same side (Pl. II, fig. III). The conidia are borne singly at the tips of the branches of conidiophores (Pl. II, figs. VI, VII). Sometimes two or more conidia may be borne on a single conidiophore.

As this species differs materially from *A. Kennedyae* McAlp. and *A. Davisiae* McAlp., it is regarded as a new species, with the following description:

Amphichaeta Punicae n.sp. Acervuli black, erumpent, scattered, conidia somewhat spindle-shaped, 5-septate, measuring 20–27 × 7–11 μ, the four inner cells dark coloured, the two terminal ones hyaline, each end cell bearing one-cilium. Apical and basal cilia similar, 6–10 μ long and both sharply curved, usually on the same side.*

Hab. On twigs of young plants of *Punica granatum* L. at Lahore, India.

* *Amphichaeta Punicae* sp.nov. Acervulis nigris erumpentibus, sparsis; conidiis aliquantum fusiformibus, 5-septatis, 20–27 μ longis, 7–11 μ latis, cellulis medianis quatuor fuscis, cellulis terminalibus hyalinis 1-ciliatis; ciliis apicalibus basilibusque similibus, 6–10 μ longis valde curvatis plerumque ad idem latus versis.

The surface of an infected twig was sterilised by rubbing with alcohol. An acervulus was then picked up, and crushed in a tube containing sterilised water, from which dilution cultures were made in Anar extract agar*. Plates were incubated at 28° C. As soon as the colonies appeared, these were transferred to plates containing different media, which were examined when fruit bodies had formed. Pure cultures were thus easily obtained.

The germination of the spores was studied in hanging drop cultures in water. Spores germinated after eight to fourteen hours at 26° C.; they swell up by absorbing water, and the cell which gives out a germ tube enlarges and draws nourishment from the adjoining cells. Both the dark and hyaline cells may give out germ tubes. Occasionally two germ tubes emerge from the same cell (Pl. II, fig. iv). The germ tube is hyaline and soon begins to branch. Though no acervuli were formed in the hanging drop, plenty of chlamydospores were formed after six weeks (Pl. II, fig. v). If instead of water, hanging drops of Anar extract are used, germination is more rapid and growth is more profuse.

The spread of the fungus in different media, the effect of different concentrations on growth, the effects of light and darkness, of aeration, of humidity, etc., were studied. Growth of the fungus was observed both in vegetative and synthetic media. Pieces of mycelium from the growing region on agar plates were transferred to the centre of Petri dishes containing various media. These were incubated at 26° C. Measurements of the diameter of the colonies were taken every second day. Table I shows the spread of the fungus in millimetres as well as other growth characters when grown on various media.

The set of inoculated Petri dishes containing different media was kept for seven weeks in order to observe the appearance of fruiting bodies. Table I shows that the spread of the fungus varies considerably, being greatest in Anar extract agar (92 mm.) and least in nutrient agar (29 mm.). Fruit bodies also vary very much in size. In such culture media as water agar, Robinson's medium and nutrient agar, acervuli are absent, whereas in Czapek's synthetic medium and potato extract agar, acervuli were found only on older mycelium in the centre. In Raulin's medium, immature acervuli were formed after three weeks. In the remaining culture media, such as Anar extract agar, rice and maize starch agar, potato glucose agar, Cook's and Richards's media, these were found in groups.

Chlamydospores were observed in potato glucose and Cook's synthetic media.

Cilia are generally of equal length at both ends of the spores, but

* Anar extract agar, prepared by cooking 100 gm. of Anar (pomegranate) in a litre of distilled water in an autoclave at 1 atmosphere pressure for 20 min., after which it is strained through muslin and made up to 1000 c.c. To this 22·5 gm. of agar is added and the medium is then autoclaved in the usual way.

Table I

Medium	Growth 18 days in mm.	Appearance of acervuli	Acervuli	Spores without cilia	Cilia	Diameter of hyphae	Zone
Anar extract agar	90	5th week, in groups, few	482–206 × 413–206 μ Av. 344 × 289 μ	29·1–22·1 × 8·8–7·6 μ Av. 24·3 × 8·2 μ	9·5–4·1 μ Av. 5·69 μ	2–4 μ	+
Rice	74	7th week, very few, scattered	550–344 × 482–206 μ Av. 454 × 303 μ	28·4–25·3 × 7·6–5·69 μ Av. 27·5 × 6·6 μ	9·5–3·8 μ Av. 6·9 μ	2–5 μ	+
Maize	82	6th week, few pycnidia	413–165 × 344–165 μ Av. 248 × 220 μ	25·3–22·1 × 9·5–6·9 μ Av. 24·6 × 7·6 μ	6–4 μ Av. 3·16 μ	3–6 μ	–
Potato glucose	81	4th week, in groups	688–275 × 482–275 μ Av. 482 × 385 μ	28·4–22·1 × 6·9–5·69 μ Av. 25·3–6·05 μ	11·1–7·9 μ Av. 9·2 μ	1–3 μ	+
Potato extract	68	6th week, very few, on the older portions only	688 × 413 μ Single	25·3 × 6·3 Av. 25·3 × 6·3 μ	6·32–5·1 μ Av. 5·6 μ	2–4 μ	–
Cook's medium	67	3rd week, comparatively much more numerous than the rest and appear very early	756–344 × 619–344 μ Av. 550 × 482 μ	28·4–25·3 × 6·9–5·1 μ Av. 26·9 × 6·0 μ	6·9–3·16 μ Av. 6·3 μ	2–5 μ	+
Czapek's	73	5th week, one unripe acervulus	Absent	Nil	Nil	1–3·9 μ	+
Raulin's	78	3rd week, few unripe only	344–275 × 302–247 μ unripe	Nil	Nil	2·6–6·3 μ	+
Nutrient agar	29	Nil	Absent	Nil	Nil	1–3·8 μ	–
Richards's	66	4th week, in few groups only	688–275 × 688–275 μ Av. 468 × 668 μ	26·9–23·7 × 6·9–6 μ Av. 25·3 × 6·32 μ	6·9–4·7 μ Av. 5·69 μ	1·5–4·5 μ	–
Robinson's	78	Nil	Absent	Nil	Nil	2·3–5·7 μ	–
Water agar	50	Nil	Absent	Nil	Nil	2–4 μ	–

sometimes the difference in length is very great. Their position in relation to the hyaline base is variable; they may be set at different angles and sharply curved. The longest cilia are found on the spores formed on potato glucose. Generally the spores of this fungus are quite big, but on maize starch agar they are small and thin.

A few hyphae running together to form coremial strands is of common occurrence and the growth is zonal in a few cases only, as for example in Anar extract, rice agar, Raulin's, Cook's and potato glucose media.

Effect of different concentration of media on growth. For this experiment only Cook's synthetic medium was used. 15 c.c. of different concentrations of Cook's medium were prepared and poured in sterilised Petri dishes. After inoculation these were kept at the room temperature with the day and night variations. The concentrations used were $N8$, $4N$, $2N$, N, $N/2$, $N/4$, $N/6$ and $N/8$. The rate of spread of the fungus was studied for over a fortnight, but no difference either in the rate of spread or in the total spread was found at the end of eighteen days. The vegetative growth in higher concentrations was quite characteristic. Pl. II, fig. x shows the striated growth seen in $8N$ concentrations. It was found that the largest number of acervuli was formed in Cook's normal medium.

Effect of light and darkness on growth. Minute pieces of mycelium were transferred to the centre of both vegetable and synthetic media in order to study the effect of light and darkness. Of the four sets of Petri dishes (two sets of Richards's and two of glucose) two sets (one set of Richards's and one of glucose) were wrapped in thick black paper and kept in continuous darkness. Diameters of colonies were measured every second day. Petri dishes kept in darkness were measured in red light inside the dark room. The following table shows the growth measurement in millimetres.

Table II

Medium	Effect of	Days 2	4	6	8	10	12	14	16
Richards's	Light	7	16	24	30	39	43	45	45
Richards's	Darkness	7	19	25	36	45	54	60	68
Potato glucose	Light	9	23	36	46	57	66	75	82
Potato glucose	Darkness	5	23	34	46	60	69	82	90

The results show that more vegetative growth takes place in darkness than in light. In Richards's medium clear zonation occurs in the dark, but in light, zonation is not so distinct. In potato glucose agar zonation occurs both in light and darkness, but in the light the rings are more compact. In light, fruit bodies were not produced but were formed in darkness.

Effect of aeration. For studying this, three sets of four agar plates

were inoculated with the fungus by placing small bits of mycelium from a pure culture in the centre of the Petri dishes by means of a sterilised needle.

One set was sealed with wax so that only limited air was available for the growth of the fungus. The other two sets were also sealed after pouring 18 c.c. of 33 per cent. KOH solution of potassium hydroxide, and 15 c.c. of pyrogallate of potash solution respectively into the lids of the inverted dishes. In the former, growth took place in the absence of carbon dioxide, and in the latter, oxygen present inside the Petri dishes was absorbed by the liquid. These were incubated at 29° C. The following table shows the growth measurement taken in millimeters.

Table III

		Days			
	Effect of	2	4	6	8
I set	Limited air	14	24	36	39
II set	Absence of CO_2	15	20	22	22
III set	Absence of O_2	Nil	Nil	Nil	Nil

From the above table, it will be seen that growth in ordinary air containing carbon dioxide is more than in air without carbon dioxide. It seems that the presence of carbon dioxide, though in a small quantity, is beneficial to the fungus. The authors found the same sort of result with another fungus causing a disease of the pomegranates studied by them.

In the absence of oxygen no growth could take place.

The upper thermal death point was also determined by following the bacterial method and was found to be between 47 and 48° C. for an exposure of ten minutes.

Inoculation experiments. Perfectly healthy plants were procured from a local nursery. These were kept under observation for over a month before inoculation.

Two plants were kept as controls and others were inoculated either by injection of spore suspensions by means of a hypodermic syringe or by prick inoculation or by spraying with spore suspensions. Three plants were inoculated in each way. A number of cut twigs and leaves was similarly inoculated. Young twigs were cut under water and the cut twigs and leaves were placed in sterilised glass vessels and Petri dishes containing cotton-wool soaked in water or wet filter papers. In all the experiments, before inoculation, the surface of the leaves and twigs was sterilised by rubbing with alcohol. The plants in pots were watered daily and examined regularly. Numerous acervuli were noticed after the third week on the leaf pricked and inoculated with a drop of spore suspension (Pl. II, fig. IX).

The following table shows the result of inoculation experiments:

Table IV

Material	Date of inoculation	Method of inoculation	Remarks on 30. iv. 32
Cut leaves	25. iii. 32	Prickings	Numerous acervuli formed after 4 weeks
,,	,,	Spraying	No effect
Cut twigs	25. iii. 32	Injections	Abundance of mycelium inside the vessels but no acervuli formed
Plants in pots	25. iii. 32	Spraying	No effect
,,	,,	Injections	No acervuli observed, though hyphae seen inside vessels*

* Numerous fruit bodies were present when the material was examined after the summer recess in the following September.

It therefore appears that *Amphichaeta Punicae*, is a weak parasite. It can infect the host only through wounds. Spraying with spore suspension failed altogether to cause infection. Injection methods, though successful, take a long time to show the formation of acevuli, though, when sections were cut, hyphae were observed inside the tissues within a fortnight. Acervuli took a long time to appear on the injected plants in pots; the cut twigs decayed before the acervuli could appear.

Summary

A disease of the pomegranate from Lahore, has been described. The fungus causing it is a weak parasite, attacking only nursery plants through wounds. Though the infected plants are not actually killed, they become very much stunted in growth.

As this species differs considerably from known species of *Amphichaeta*, it has been described as a new species.

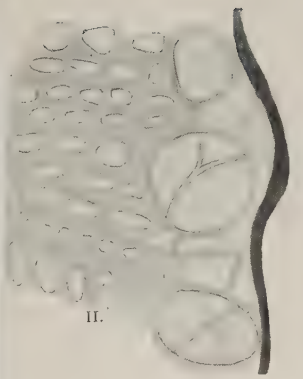
The spores of *A. Punicae* germinate readily in eight to fourteen hours and the fungus will grow on various media. The behaviour of the fungus on different media has been studied as well as the effect of different external factors on its growth.

Though it is a weak parasite, its pathogenicity has been established by means of a large number of inoculation experiments.

EXPLANATION OF PLATE II

Fig. I. Twig showing pycnidia. $\times \frac{3}{8}$.
Fig. II. Transverse section of twig showing hyphae in the xylem vessels. $\times 510$.
Fig. III. Spores of *Amphichaeta*. $\times 510$.
Fig. IV. Germinating spores in hanging drops of water. $\times 510$.
Fig. V. Chlamydospore formation in hanging drops, two months' old culture. $\times 620$.
Figs. VI and VII. Conidiophores bearing conidia. $\times 510$.
Fig. VIII. Transverse section of a pycnidium. $\times 120$.
Fig. IX. Control and inoculated leaves. The latter bearing numerous pycnidia. $\times \frac{3}{4}$.
Fig. X. Photograph showing character of growth in 8*N* concentration of Cook's medium. $\times 1\frac{7}{12}$.

I.



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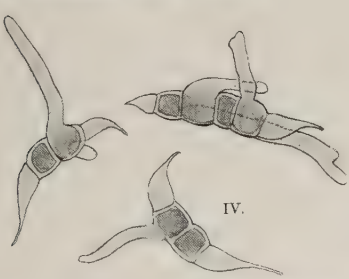
III.



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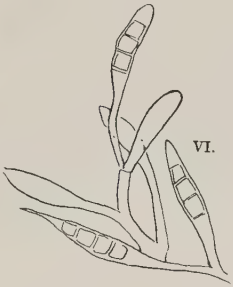

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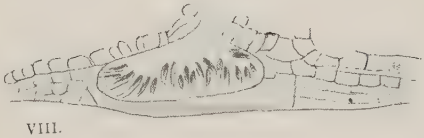
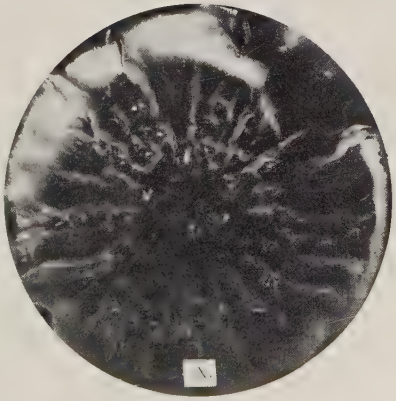


VIII.

IX.



X.

A BRIEF ACCOUNT OF FUNGI PRESENT IN THE AIR OVER ORCHARDS, WITH ESPECIAL REFERENCE TO *PLEOSPORA* AND *POLYOPEUS*

By F. M. CARTER
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(With 1 Text-figure)

Introduction

In an investigation on the distribution of micro-organisms in the air, Horne and Nitimargi conducted a systematic exposure of plates of nutrient medium at various localities and made a statistical analysis of the organisms which later developed on the plates. A preliminary account of this investigation has already appeared (8, 9).

This paper deals with the identity of the fungi thus obtained from the air over orchards at East Malling Research Station; at the Horticultural College, Swanley; and in the vicinity of Belfast, Northern Ireland. It forms, in conjunction with the work of Horne and Nitimargi, an attempt to gain further knowledge as to the extent to which decay of apples in storage may be attributed to fungal infection from the atmosphere before the gathering of the fruit.

Special attention has been paid to *Pleospora* and *Polyopeus*, since these fungi, which are definitely known to cause disease of apples, were of such frequent occurrence on the plates.

Culture of the fungi

The method of trapping the organisms has been fully described elsewhere (8). Individual fungi were isolated from the collection on the exposed plates by subculturing from distinct colonies after a few days' growth.

When bacterial or other contamination was present subculturing on to plates of plain agar was repeated, taking very small inocula from the periphery, until an apparently pure growth was obtained.

It was considered that, for the purpose of this investigation, the advantage of taking monohyphal tip cultures did not justify the labour involved, but monospore isolations were made from a few of the fungi, such as *Pleosphaerulina*, which produce large spores.

Cultures were, in general, maintained on a standard synthetic medium (2·0 gm. asparagine; 0·75 gm. $MgSO_47H_2O$; 1·25 gm. K_3PO_4; 2·0 gm. glucose; 10 gm. starch; 15 gm. agar; 1000 c.c.

water). When reproduction did not occur on this medium potato mush agar, malt extract agar, oatmeal agar and Crabill's medium (5·0 gm. $MgSO_4$; 1·0 gm. K_2HPO_4; 2·0 gm. $NaNO_3$; 0·5 gm. KCl; 0·1 gm. $FeSO_4$; 10 gm. potato starch, 20 gm. agar; 1000 c.c. water) were tried.

Identity of the fungi

Several thousand fungal colonies appeared on the exposed plates in the course of experiment and further culture of the whole number was clearly impracticable but an effort was made to isolate the fungus from at least one colony of each of the different types which occurred.

Identification of the fungi presented a somewhat difficult problem. Lack of knowledge of the natural host, inability to produce ready sporulation, and variation of the organism under cultural conditions all combined to render it rarely possible to state more than the generic position of the fungus.

A list of the genera represented in the collection, with the specific name where possible, is given below.

Nitimargi, who made a preliminary grouping of the cultures into genera, tested many of these fungi on Worcester Pearmain apples, and an asterisk is placed against the name of those which showed an ability to attack the fruit.

Species known to be associated with disease of apple fruit, or, where the specific name is lacking, genera containing such species, are indicated by the addition of references to records of such disease.

List of fungi obtained from the air over orchards

Phycomycetes

Mucor (5, 11), *Rhizopus* (1, 5, 7, 11), *Zygorhynchus*.

Ascomycetes

Rosellinia, *Chaetomium*, *Pleosphaerulina hyalospora*, **Pleospora* spp. (5, 10), *P. herbarum* Pers.? (10, 16), *Melanospora leucotricha* Corda.

Fungi Imperfecti

Sphaeropsidales: *Phomopsis*, *Phoma* (1, 5, 10, 11), *Macrophoma*, *Polyopeus purpureus latirostratus* Horne, *P. purpureus* Horne—other strains (11), **Polyopeus* spp. (10), **Sphaeropsis malorum* Peck, **Coniothyrium* (5, 10, 11), *Ascochyta* (7, 11), *Diplodia* (11).

Melanconiales: **Pestalozzia Hartigii* Tub.

Moniliales: *Oospora abortifaciens* Quek., *Monilia*( ), *Aspergillus* (11), *Penicillium* (1, 5, 7, 11), *Scopulariopsis brevicaule* Link, **Eidamia* (7, 11), **Sporotrichum roseum* Link, **Botrytis cinerea* Pers. various strains (5, 11), **Verticillium lateritium* Berk., **Cladosporium* (7), *C. herbarum* Pers.(?) (11),

**Stemphylium Berlesii* Oud., *Macrosporium* **Alternaria* spp.(10, 11) including *A. tenuis* Nees.(?)(5, 11), *A. humicola* Oud.(?) *Stysanus stemonites* Corda(11), **Epicoccum granulatum* Periz.(5), *Fusarium* spp.(5, 10, 11) including **Fusarium culmorum* (W. G. Sm.) Sacc.(11), **Fusarium fructigenum* Fr.(11), **Trichothecium roseum* Link(1) (*Cephalothecium* Corda).

Observations on *Pleospora Herbarum* pers.

A number of different strains of *Pleospora* were obtained from the exposed plates and were identified at the British Museum (Natural History) as strains of *P. herbarum* Pers.

Ellis(3) and Rose and Butler(16) record a *Macrosporium* conidial stage of *Pleospora herbarum*; Machacek(12) has identified *Macrosporium parasiticum* and *Pleospora herbarum* as stages in the life history of one fungus which causes spot on onion stems, and Kidd and Beaumont(11) have stated that the conidial stage of the *P. herbarum* which they found on apples was *Macrosporium commune*. The conidia produced in the early cultures of the strains now under consideration were of the *Stemphylium* type, but they ceased to be formed before their connection with the perfect stage had been established.

Perithecia were formed in abundance in most of the cultures, but the proportion which proved fertile was low. Some strains never produced ascospores but were placed in this group on account of their general appearance and the presence of black bodies resembling immature perithecia.

Some of the originally fertile strains became sterile later, possibly as a result of repeated subculture on the standard medium, since Newton(13) refers to the tendency of other species of *Pleospora* to lose the power of producing perithecia when cultures on a rich medium are frequently renewed.

Table I summarises the cultural characters of the various strains of *P. herbarum* investigated. Since cultures of the different strains were most distinct from each other on the standard medium but most fertile on oatmeal agar, descriptions of vegetative characters relate to standard medium cultures while perithecial characters are those observed on oatmeal agar.

The descriptions of the colours produced in the medium follow Ridgway's system of nomenclature(15).

The reaction of a number of the strains to addition of acid to the medium has been dealt with elsewhere(2). The radius of spread of each strain after nine days' growth at 20° C. in standard medium containing 0·0, 0·25 or 0·5 per cent. malic acid is given in Table II.

The strains were also tested on Worcester Pearmain apples. Inoculation was performed as described by Granger and Horne(4). Each strain was used to inoculate ten apples and each apple was

Table I. Pleospora Herbarum, *Strains* 1–16. *Vegetative characters on*

Strain	Colour produced in standard medium	Colour produced in oatmeal agar	Outline of growth	Colour of mycelium	Type of growth
1	Yellowish	None	Regular	White	Silky aerial mycelium with even surface
2	,,	Pale yellow-orange	,,	,,	Woolly aerial mycelium with uneven surface
3	,,	,,	,,	,,	Prostrate in parts. Woolly aerial mycelium snow-like appearance
4	,,	Honey-yellow	,,	Greyish white	Woolly, snow-like. Darker at centre
5	Yellowish with some black	Pale yellow-orange	,,	White to greyish	Short, woolly. Thicker at centre. Margin transparent
6	,,	Honey-yellow	,,	,,	Fluffy at centre, less thick at margin
7	,,	Pale yellow-orange	Irregular	,,	Slight, hirsute growth
8	Pinkish yellow with some black	Ox-blood red	Regular	Greenish grey	Short, silky mycelium. Centre darker than margin
9	,,	,,	,,	,,	Erect, silky. Centre darker than margin
10	,,	None	,,	Grey	Short, woolly mycelium
11	,,	Ox-blood red	,,	Greyish white	Compact. Margin transparent
12	Pinkish yellow with some black at centre	Salmon-pink and pale orange-yellow	,,	,,	Spreading. Fluffy in patches. Thicker at centre
13	Pinkish yellow with some black	Ox-blood red	Indefinite first. Regular later	,,	Spreading. Fluffy in patches
14	,,	None	Lobed	,,	,,
15	Orient-pink	Salmon-pink and pale orange-yellow	Regular	White	Silky mycelium. Margin transparent
16	,,	Ox-blood red and vinaceous	Stellate	,,	Silky mycelium almost prostrate. Narrow transparent marginal zone

on's synthetic medium. Perithecial characters on oatmeal agar

Zoning	Conidia	Perithecia	Ascus dimensions in μ	Ascospores					Strain
				Length in μ	Width in μ	Trans. divis.	Long. divis.	No. per ascus	
—	—	Numerous sterile	—	—	—	—	—	—	1
dications	—	Numerous 472–555 μ diam.	203×29	36	12·5	7 or 8	2	8	2
—	—	Numerous sterile	—	—	—	—	—	—	3
—	*Stemphylium* type	,,	—	—	—	—	—	—	4
—	—	Numerous 580 μ diam.	180×32	34	13	7 or 8	2	8	5
dications	—	Numerous. Few fertile 520 μ diam.	176×25	35	12·7	7	1 or 2	8	6
—	—	Numerous 480 μ diam.	125×23	34	12	7	1	8	7
dications	—	Numerous sterile	—	—	—	—	—	—	8
—	—	Numerous. Very few fertile 360–720 μ diam.	300×28	36	13·5	7	2	8	9
arrow	—	Few sterile	—	—	—	—	—	—	10
—	—	Numerous sterile	—	—	—	—	—	—	11
—	—	,,	—	—	—	—	—	—	12
—	—	,,	—	—	—	—	—	—	13
—	*Stemphylium* type	Numerous 380×260 μ	117×26	35	14	7	2 or 3	8	14
—	,,	Numerous sterile	—	—	—	—	—	—	15
—	—	—	—	—	—	—	—	—	16

inoculated with three strains, at different equatorial points. All the strains tested were found capable of attacking the fruit. The rot was estimated twenty days after inoculation by weighing the apple before and after removal of the decayed tissue from each infected area in turn. From the data obtained, the radial advance of each strain was calculated and the resulting values are also shown in Table II.

It will be observed that the strains differ in activity both in synthetic media and in the apple fruit.

Table II. Pleospora herbarum. *Relative activity of thirteen different strains in apple fruit and in standard medium varying in malic acid content*

Strain	Radial advance in apple fruit (mm. after 20 days at lab. temp.)	Radius of spread (mm. after 9 days at 20° C.)		
		0·0 % acid	0·26 % acid	0·5 % acid
10	2·0	34·0	27·5	18·0
9	1·8	36·0	25·0	22·0
12	1·1	33·5	14·0	9·0
7	1·0	25·0	16·0	12·5
2	1·0	34·0	29·5	22·5
5	0·9	30·5	20·5	14·5
16	0·9	35·0	26·5	17·0
15	0·9	37·0	13·5	17·5
1	0·6	30·0	16·0	12·0
3	0·5	29·0	21·0	13·5
4	0·5	31·5	21·0	14·5
8	0·4	32·0	17·5	19·5
13	0·3	20·0	12·5	6·5

Observations on *Polyopeus* spp.

Colonies of *Polyopeus* appeared on nearly every exposed plate. Many were easily recognisable as strains of *P. purpureus* Horne (6); others could not be placed with certainty in any existing species. Several of the latter forms, numbered for convenience *Polyopeus* 1–8, were grown on Crabill's medium in which pycnidia rapidly developed. The various types of pycnidium are illustrated in Fig. 1, while the cultural characters of the fungi are given in Table III.

It will be observed that the pycnidia differ considerably in size, shape and colour, and the necks are diverse in colour and appearance but any particular culture was consistent in the type of pycnidium it produced.

Polyopeus 2 has been grown on media varying in carbohydrate and malic acid content as described in another paper (2), where it is shown that acid retards growth to an extent dependent on the concentration of carbohydrate. Data illustrating this point are reproduced in Table IV.

Table III. Polyopeus *spp.* *Characters on Crabill's Medium*

Polyopeus	Colour produced in medium	Colour of mycelium	Nature of mycelium	Chlamydospores	Pycnidia: Distribution of Pycnidia	Pycnidia: Depth in medium	Pycnidia: Dimensions of body (in μ)	Pycnidia: Colour of body	Pycnidia: Type of opening	Pycnidia: Number of openings	Pycnidia: Colour of neck	Pycnidia: Dimensions of neck (in μ)	Length of hyaline spores (in μ)	*Polyopeus*
1	Very slight blackening	White to brownish white	Sparse, superficial	None	Scattered over surface, separate	Seated on surface	148×130 to 259×240	Black	Neck sometimes slightly curved	1 or 2*	Brown	110×93	4·6	1
2	None	White	,,	,,	Scattered over surface, mostly separate	Slightly embedded	167×93 to 225×185	,,	Slender necks, straight or bent	1 usual, occasionally 2 or 3	Black	Up to 167×55	5·2	2
3	,,	,,	Superficial film	,,	Scattered over surface, separate except at limit of culture where they are crowded together	,,	Up to 400×400	Hyaline-brown-black	Necks lying parallel to surface	1–7	Dark brown or black	Up to 370×95	4·5	3
4	Purple	,,	Fluffy growth which may cover pycnidia	,,	Scattered over surface, usually single, some aggregated into large compound bodies	,,	220×200 to 440×370	Hyaline	Short neck or ostiole	1 usual. Multiple ostioles where pycnidia join	Black	Ostiole diam. 40–50	6·8	4
5	Black	,,	Sparse, aerial	Numerous brown unicellular	Aggregated into large, irregular groups all over surface	Base slightly embedded	Single 185×185. Group of 8, 460×370	Hyaline-light brown	Irregular necks, bent, occasionally branched	1–several	Hyaline at base. Black at tip	Length up to 315. Width up to 130	4·3	5
6	Grey to black	,,	Sparse growth over surface	Some at bottom of tube	Scattered over surface, single or aggregated to form large, compound bodies	Seated on surface	185×185 to 315×240	Hyaline	Short necks or ostioles	Openings may be confluent where pycnidia join	Black	55×46. Ostiole diam. up to 55	4·3	6
7	Black	Greyish white	Thickly woven layer over surface	Numerous	Scattered. Not very numerous	Sunk in medium	130×90 to 260×90	Black with lighter areas	Indication of neck each end	1 or 2	Black. Lighter at tip	—	No spores	7
8	,,	White	Thin malted growth	,,	Few, undersized	,,	—	Black	Slight indication of short wide neck	1 or 2	Slightly less dark than rest of pycnidia	—	No spores	8

* One neck found with two openings, see Fig. 3.

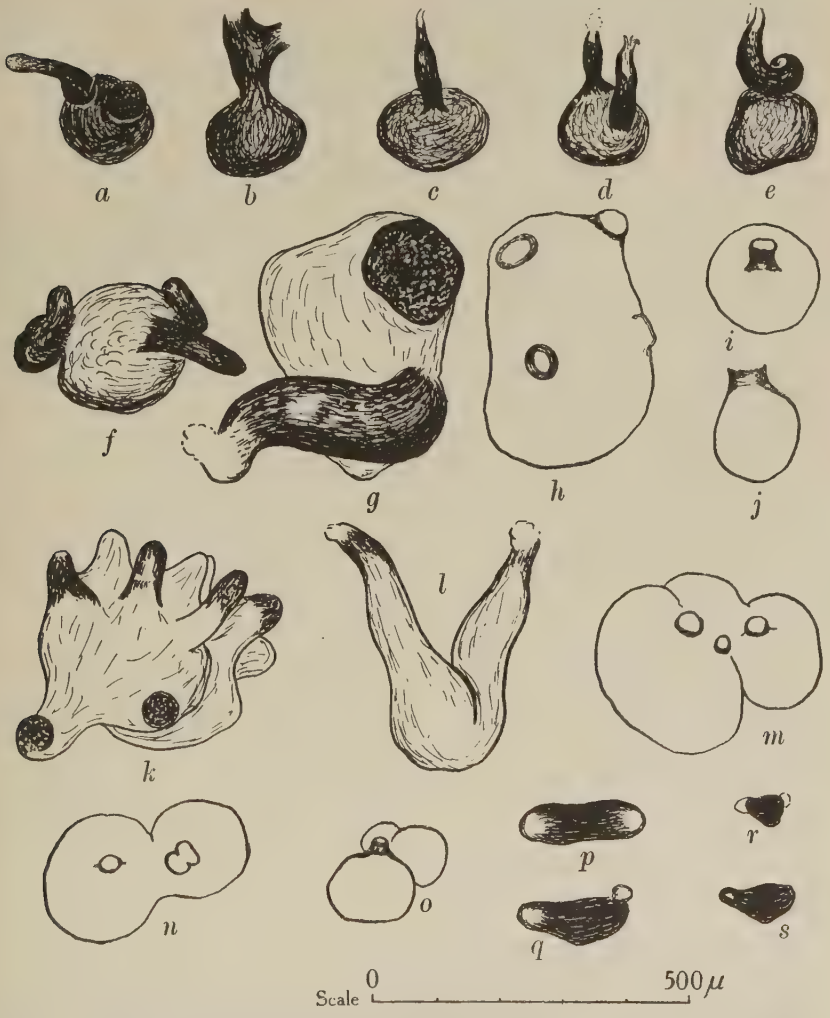

Fig. 1. *Polyopeus* spp. Pycnidia formed on Crabill's medium. *a*, *b*, *Polyopeus* 1; *c*, *d*, *Polyopeus* 2; *e*, *f*, *g*, *Polyopeus* 3; *h*, *i*, *j*, *Polyopeus* 4; *k*, *l*, *Polyopeus* 5; *m*, *n*, *o*, *Polyopeus* 6; *p*, *q*, *Polyopeus* 7; *r*, *s*, *Polyopeus* 8.

Table IV. Polyopeus 2. *Radius of spread (mm.) after nine days' growth in media varying in carbohydrate and malic acid content.* 20° C.

Carbohydrate		Malic acid %		
Nature	%	0·0	0·3	0·6
Glucose	0·2	27·0	21·0	13·0
,,	9·0	44·0	20·0	11·0
,,	12·0	26·0	11·0	10·0
Sucrose	17·1	20·3	8·0	0·0

Polyopeus is one of the commonest causes of "spots" which develop in apple fruit at a late period in the season (7, 10, 11), and it is worthy of note that all the strains mentioned have been proved to be capable of developing in Worcester Pearmain apples.

Discussion

The range of genera represented by the fungi which settled from the air over orchards is wide but, as pointed out by Horne (8), the colonies which appeared on the exposed plates do not necessarily include every species falling on the plates. Some fungal spores may be unable to develop on the particular medium used, others may be incapable of growth on any artificial medium, while others again, may require a period of rest before germination.

These facts probably account for the absence of Basidiomycetes and the preponderance of Fungi Imperfecti which, in general, are tolerant of most media.

It is noticeable that all but a few genera on the list have been recorded as occurring on apple fruit, but it must not be assumed that the unnamed species belonging to genera which contain some pathogenic members, are, without exception, those species which are responsible for disease.

It is also interesting to note that some species such as *Sporotrichum roseum* and *Verticillium lateritium*, although not previously isolated from apples, have been found capable of attacking the fruit when artificially introduced.

While most of the fungi listed in this paper are known to occur on apples, the converse is also true, that the majority of the fungi isolated from diseased apples at all stages of maturity appear on the list. Hence it seems reasonable to conclude that apple rots in storage, are, to a great extent, due to fungal infection from the air before picking.

Horne (8) has shown that the number of fungi present in air over an apple orchard at Exning, Cambridgeshire, far exceeds that in air over orchards at East Malling. He also points out that the East Malling crop suffers much less from fungal attack in storage than does that from Exning, although resistance to fungal invasion after artificial inoculation is actually less in East Malling apples than in those from Cambridgeshire. Further evidence in this paper of the pathogenic nature of the fungi obtained from air over orchards supports Horne's suggestion that absence of infection rather than peculiar powers of resistance may account for the freedom from storage disease shown by apples from localities such as East Malling.

Summary

A list is given of fungi which settled on plates exposed to the air over apple orchards, together with references to records of their pathogenicity towards the apple fruit.

Several strains of *Pleospora herbarum* Pers. are described and some forms of *Polyopeus* Horne which could not be ascribed to any known species are briefly recorded.

It is suggested that disease of apples in storage is chiefly due to fungal infection from the atmosphere before the gathering of the fruit.

The author wishes to thank Dr A. S. Horne for his help throughout the work, Prof. V. H. Blackman for his interest and criticism, and Miss F. L. Stephens, British Museum (Natural History), South Kensington, for kindly identifying a number of the fungi.

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NOTE ON THE ISOLATION OF SINGLE SPORE CULTURES

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(With 1 Text-figure)

The difficulty of isolating single spores has been appreciated by mycologists, and small spores, such as the conidia of *Eurotium herbariorum*, provide special difficulty. During a study of the cytology and physiology of this fungus, it became necessary to investigate the phenomenon of aversion.

For this purpose single spore cultures were essential. A simple technique was developed to isolate single spores of *Eurotium* and this method, described below, was found to be effective, the chances of contamination being practically obliterated.

Details of the technique

Capillary tubes (diameters 0·5–0·75 mm.) were drawn from clean glass tubing. Each capillary tube was drawn to a very fine aperture at one end and sealed off; the other end was also sealed by quickly putting it into the flame and drawing rapidly.

The resulting capillary tube (Fig. 1 *a*) was perfectly sterile, the total length of the tube being ten to twelve inches. The tube was then bent in the middle at an angle of from 30 to 45° by rapidly passing it through the flame of a fish-tail burner.

The capillary tubes are difficult to bend in a large flame, in which they may collapse. Dr B. Barnes has therefore suggested the use of a "micro-flame" which consists of a glass tube with a jet having a very fine aperture. This tube is attached to the gas outlet with a length of rubber tubing. The small flame (="micro-flame") is extremely useful, both in closing the ends of the capillary tubings and bending them.

A spore suspension was made in a test-tube containing sterilised water. The outside of a capillary tube was sterilised by means of alcohol over the surface (with care, these tubes can be sterilised in the auto-clave). As soon as the alcohol had evaporated the test-tube was opened and the *A* end of the tube (Fig. 1 *a*) immediately pushed into it and the plug was replaced. This end was broken by tapping the tube against the bottom of the test-tube. As soon as the tube was

broken at the other end the suspension rapidly travelled up, and completely filled the tube, but the free end being so fine, the suspension could not drop (Fig. 1 *b*). When this end was touched to any surface, a tiny drop emerged. On examining drops on slides under the microscope, it was found that most of them contained a single conidium.

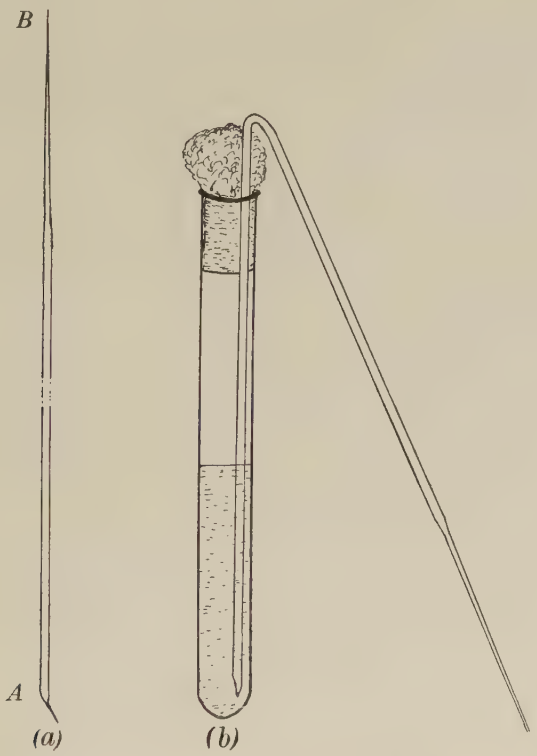

Fig. 1. (*a*) Capillary tube closed at both ends *A* and *B*. After bending the end *A* is put in test tube; both ends are broken. (*b*) Test-tube containing conidial suspension in sterilised water. Capillary tube is in position, and the apparatus is ready for sowing suspension drops.

Petri dishes containing sterilised agar-agar were marked underneath with ink-dots. The suspension drops were sown over these dots and the dishes were incubated at 33° C. to favour germination. Isolation of individual conidia before germination was found to be practically impossible (search for them being in vain).

Later, it was found that the usual medium (prune agar) was more

satisfactory than pure agar for sowing the suspension drops as germination was more rapid upon it and further growth more satisfactory. After twenty-four to thirty hours the dishes were examined under the microscope and single germinated conidia were isolated with the help of a dummy objective*.

To minimise the chances of contamination it was found useful to sow a single suspension drop in the centre of a Petri dish and isolate the young germinated conidia.

The drop spreads out quickly, so that if it contains more than one spore they separate out and give sufficient room for isolation.

The free end of the capillary tube was sterilised with absolute alcohol every time it was used.

* De la Rue, C., "Isolating single spores." *Bot. Gaz.* LXX (1920), 319.

PROCEEDINGS

Meeting held at University College, London, March 17th, 1934, Dr B. Barnes, President, in the Chair.

E. M. Blackwell. The Germination of Oospores of *Phytophthora Cactorum*.

After a citation of the few definite records of germination of oospores of *Phytophthora* spp., a method of refrigeration was described by which profuse germination of oospores of *P. Cactorum* had been obtained at will during the winter 1933–4.

A possible explanation was then offered of the action of the various mechanical, physical and chemical agencies that have in the past been used to induce germination of resistant spores of fungi, viz. cracking of the spore membrane, heat, cold, drought, "nutrients," volatile substances, acidity, CO_2.

The following facts were considered:

(1) That a resting spore is a colloidal complex of protoplasm and reserves enclosed within an almost impermeable membrane of fungus cellulose also colloidal in structure;

(2) that during dormancy of the spore these colloids are at their iso-electric point: the point at which they hold a minimum of "bound" water;

(3) that it is upon the enclosing membrane that the various agencies work: the protoplasm and reserves coming into play later when germination is initiated and the conditions for germination secured.

It was suggested that the effect of the physical agencies upon this resistant membrane would be to bring about a permanent change in the configuration of its colloidal system, thus forming cracks through which water and gases could pass; and that the various chemical factors might all bring about a change in the *p*H of the colloids thereby inducing swelling and so producing intermicellar spaces through which water and gases could pass.

Since many observers have found that a period of "after-ripening" is enough in itself to induce germination, it was further suggested that since CO_2 tends to accumulate within a resting spore there would ultimately come a moment when this would add an electric charge to the colloids and the resistant wall might be rendered permeable *from within*.

E. H. Ellis. Preliminary Observations on Mycorrhiza.

Continuous field and laboratory observations over long periods on the seasonal variation of the mycorrhiza of *Pinus sylvestris* and *Larix decidua* suggest that the association of tree and fungus is obligate to the trees beginning in *Pinus sylvestris* in the seedling stage. Experimental addition of a mixed infection produced a marked increase in growth rate resulting in the production of giant seedlings. So far as can be ascertained, infection of a young root normally occurs during late autumn when the mycelia of Basidiomycetes are active and small brown knots of neoplasmic growth are formed on the roots. In the following spring when growth of tree roots in the humus layer once more becomes active, these knots are enlarged and become bright pink. During summer the close knot of rootlets is separated by a rapid longitudinal growth giving rise to the coralloid root that has so often been described, and in early autumn this phase is succeeded by one in which the coralloid triangles become draped with a loose mesh of mycelium. Usually during late summer and early autumn it can be seen that the normal direction of growth of lateral roots is reversed and they grow upwards from soil into the humus layer and

there become infected by fungi. No suggestion is offered on the influences responsible. Observations of other workers are confirmed that Hymenomycetous fungi are concerned in the association. Species of *Boletus* are principally found on *Larix decidua*, but there is a more mixed range, frequently with *Marasmius peronatus*, on *Pinus sylvestris*. Animal activities, notably those of mice and beetles, are thought to be important factors conditioning the growth of the fungi. Insufficient evidence is available to understand the nature of the symbiosis, but the old distinction of ecto- and endo-trophic mycorrhiza is thought to be more descriptional than actual and may be seasonal. The fungus commonly penetrates the cortical cells of roots but seems unable to pass beyond the endodermis to the stellar region. The "Hartig net" is a pseudo-parenchyma of hyphae. The fungus mantle of larch sometimes shows on its outer surface small spines that abstract microconidia. It is hoped these may prove to be Hymenomycetous conidia.

J. B. Hamond. A Graft Disease of Walnuts caused by a Species of *Chalaropsis*.

In 1930, a fungous disease caused an almost complete failure of the walnut grafts at the East Malling Research Station. Brown spores, found in abundance on the cut surfaces of the diseased stocks and scions, were isolated in pure culture, where they gave rise to chains of hyaline endospores. These endospores, in conjunction with the brown macrospores borne singly and terminally, indicated that the walnut graft fungus belonged to the genus *Chalaropsis* of Peyronel.

Three other strains of *Chalaropsis*, isolated for comparison, were obtained from a walnut root, carrots and peach seedlings respectively.

The four strains of *Chalaropsis* all bear a general morphological resemblance to one another. When growing freely, most of the mycelium is in the form of endoconidiophores consisting of one to four cells terminating in a tapering endoconidial cell. The macroconidia are sessile, or borne on unicellular conidiophores. In slowly growing *Chalaropsis* cultures, very thin, vegetative mycelium, with intercalary swellings appears in profusion. The strain isolated from carrots may be distinguished from the other strains by its macrospores which are almost spherical, instead of ovoid, and by the fact that the endospores are olive-green when seen in mass.

The cultural relations of these fungi have been studied by growing them on various media. It was found that the fungi tend to form a series, at one end of which is the walnut graft strain, usually forming cultures which are light-coloured and regular in outline, while the carrot strain, at the other end of the series, produces typically fringed growth, which is dark green or nearly black. The other two strains are intermediate.

The type of growth obtained at different temperatures was studied for each of the four strains and differences in the response of each strain to temperature, suggested an adaptation of the fungus to the environment of its host. The type of growth obtained at various degrees of acidity was also studied, and tests were applied for the more important enzymes.

On young walnut trees, the fungus always enters through a wound or at a cut surface, and seems unable to penetrate the uninjured epidermis. Sections through diseased stocks and scions, showed spores all along the cut surfaces, and mycelium in all types of cell. Naturally infected tap roots showed varying degrees of disintegration of the tissue, beginning at the cut surfaces.

Inoculations on walnuts at the time of grafting, yielded positive results, artificially inoculated plants being indistinguishable from those which had become naturally infected. None of the strains of *Chalaropsis* resulted in much damage when inoculated into shoots of established walnut trees, all lesions eventually healing normally. Striking differences in degrees of parasitism were shown when the four strains of *Chalaropsis* were inoculated into carrots, the fungus isolated from this host being the most parasitic. When the *Chalaropsis* strains were inoculated into lupin seedlings (the host on which *C. thielavioides* was originally recorded in Italy), there

was very little damage, the plants continuing to grow normally. Throughout these series of inoculations, no injury resulted from the fungus unless the epidermis of the host was injured.

Laboratory tests in the control of *Chalaropsis* have shown that formalin is highly toxic to the macrospores. As formalin can be conveniently manipulated, it is now generally used to control the walnut graft disease at East Malling.

These four fungous strains bear a general resemblance to *C. thielavioides* described by Peyronel. Until the perfect stage is discovered, therefore, it is proposed to adopt the name *C. thielavioides* for these strains. In view of Peyronel's work on *C. thielavioides*, it seems very probable that the two fungi described under different names by McAlpine in his *Fungous diseases of stone-fruit trees in Australia, and their treatment*, should also be relegated to the genus *Chalaropsis*, and described as one fungus. Also, macrospores similar to those found on walnut grafts, have now been identified on some walnut shells in the Herbarium at Kew. Previously, endospores only had been recognised on these shells. This indicates that *C. thielavioides* was present in Britain before it was found on diseased walnut grafts.

J. Ramsbottom. Fragmenta Bibliographica.

The volumes shown were a copy of Sowerby's *English Fungi* with the original wrappers (cf. *Trans. Brit. Mycol. Soc.* xviii (1933), 167–70); a supplement to Fries's *Systema Mycologicum* (1830) (cf. tom. cit. p. 316); and two copies of Persoon's *Synopsis Fungorum*, one of these from the library of the Linnean Society of London and formerly in the possession of J. E. Smith, having a couple of cancel pages, one with the description of a genus *Bungea* afterwards replaced by *Batarrea* (photograph in *Proc. Linn. Soc. Lond.* cxlvi (1933), 15–16), the other showing that the two parts of the *Synopsis* were published as one volume.

E. M. Wakefield. Exhibit of fungi from British Guiana.

REVIEW

The Genus Diaporthe *Nitschke and its segregates*. By Lewis E. Wehmeyer. Pp. 349, with xviii plates. Ann Arbor, University of Michigan Press, 1933.

It has been generally known that Dr Wehmeyer has been engaged for some years on a monograph of *Diaporthe* and all mycologists will welcome its appearance. The author begins his preface:

"The description of new species and genera is no longer the chief purpose of systematic mycology. In many groups, particularly in the Ascomycetes, the numerous incomplete or inadequate and scattered descriptions of species and genera have accumulated as an undigested mass of material, which makes the description of new species and genera in these groups extremely hazardous. Confusion in the taxonomy and the literature also renders insecure ecological or distributional deductions from these data.

"It is extremely important, therefore, in order to advance our knowledge of the taxonomy, phylogeny, and biology of these groups and to put it on a firm basis, that there should be a careful comparative study of the collections and data already amassed, so that they may be reorganized into a workable and related whole."

With this probably everyone would agree, though many would object to the first sentence. I, for one, hold very strongly that "species-mongering" has never been the chief purpose of systematic mycology.

Monographic study of the kind here presented is an urgent need, but unfortunately few have the time or the opportunity to accomplish it. The intensive knowledge gained by going over the whole field gives a feeling of taxonomic security which otherwise is wanting. The present study is "based on material actually seen and examined." The Introduction occupies thirteen pages and deals plainly and concisely with the points at issue. From it we learn that 650 species have been ascribed to the genus *Diaporthe* founded by Nitschke in 1867 [1870]. About a hundred of these are either wrongly or doubtfully placed, and many others fall into synonymy. Some genera have been segregated and those adopted and treated in the present work are *Apioporthe*, *Diaporthopsis*, *Diaporthella* and *Cryptodiaporthe*. (*Diaporthella* was proposed by Petrak in 1924. It includes a few species characterized by the very strongly developed erumpent stroma.)

The treatment of the species is on the same lines as in the author's paper in the *Transactions* (xvii (1933), 237–95). The species are separated on purely morphological grounds and little or no weight is placed on host-relation. Dr Wehmeyer has had much experience in growing these fungi in culture and consequently speaks with authority but I feel that he has been somewhat ruthless and will not be followed in several of his suggestions. The erection of "species" in *Diaporthe* has been somewhat like that in Fungi Imperfecti—a new host a new species—with usually no attempt to point out its morphological similarities. But the account of *Diaporthe Arctii* occupies twenty pages: the species is described and one variety—the rest of the space is occupied with the description of the fungus on over twenty different hosts and the sinking of names for both the perfect and conidial stages. Similarly *D. pardalota* occupies twenty pages and *D. eres* thirty.

There can be no doubt that the author has done most valuable work in arranging so many forms under a species of wide extent. Whether he is right in assuming that the differences are merely due to the host remains for future investigations to show for we know little about the host limitation. From the grouping, clues to several problems are apparent and it is to be hoped that these will soon be followed up, for it is certain that we now have a sure foundation.

The book is well produced and has a workable index. It is worthy of a fuller and more critical notice than can be attempted here, for it gives the results of a very arduous investigation.

J. R.